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(71) Applicant *(for all designated States except US)*: **ROGER WILLIAMS HOSPITAL** [US/US]; 825 Chalkstone Avenue, Providence, RI 02908 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **REN-HEIDENREICH, Lifen** [US/US]; 62 Tiffany Road, Coventry, RI 02816 (US).

(74) Agent: **WHITE, John, P.**; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).

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(54) **Title:** BI-SPECIFIC ANTIGEN-BINDING COMPOSITIONS AND RELATED METHODS

(57) **Abstract:** This invention provides a composition of matter comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety. This invention also provides related nucleic acids, host-vector systems, compositions and methods of polypeptide production. This invention further provides related methods of treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, and kits for practicing same.

BI-SPECIFIC ANTIGEN-BINDING COMPOSITIONS AND RELATED METHODS

This application claims priority of U.S. Serial No. 60/374,930, filed April 23, 2002, the contents of which are incorporated herein by reference.

Throughout this application, various references are cited. Disclosure of these references in their entirety is hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

Background of the Invention

Defective immunity is responsible for tumor development in cancer patients. In order to use a patient's own immune system to fight cancer, a number of cell-based adoptive immunotherapy approaches have been tried (9, 11, 12, 20). These approaches include lymphokine-activated natural killer cells, tumor-infiltrating lymphocytes, auto-lymphocytes, activation of lymph node-draining T cells, antigen-specific cytotoxic T lymphocytes, anti-CD3-activated T cells, anti-CD3/anti-CD28 co-activated T cells, and dendritic cells. Although these approaches have been informative, clinical responses have usually shown no effect because of the lack of specificity toward any particular tumor.

New strategies have therefore been developed to combine the specificity of antibodies with the cytotoxic capability of T cells. The bi-specific monoclonal antibody (BsAb) approach is one of the new adoptive immunotherapy strategies.

A BsAb, in one embodiment, consists of two monoclonal antibodies (mAbs) cross-linked through chemical heteroconjugation. The BsAb will therefore carry dual specific "arms"; one arm recognizing and specifically binding to a tumor-associated antigen (TAA) and the other one recognizing the CD3 receptor on T cells. When a BsAb bridges a T cell and a tumor cell, the armed T cell can bypass the major histocompatibility complex (MHC) restrictions and become a TAA-specific cytotoxic T lymphocyte (CTL) against tumor cells bearing the TAA. *In vitro*, these BsAbs have shown specific cytotoxicity against tumors (25). In the treatment of cancer, BsAbs have improved human survival rates and eradicated tumors in animals (24).

Her2/neu is a member of the epidermal growth factor receptor family of tyrosine kinases that is over-expressed in several cancers, including breast cancer (21). A chemically heteroconjugated anti-CD3 x anti-HER2/neu BsAb was used to treat high-risk breast cancer (13, 21) and hormone refractory prostate cancer.

However, chemically heteroconjugated BsAbs have important clinical limitations (1, 22, 24, 26). First, the murine-derived mAbs induce HAMA (human anti-mouse antibody) responses in nearly all patients (5). Second, chemical heteroconjugation procedures are still inefficient and inconsistent. Third, the heterogeneous conjugation product contains a mixture of monomer, dimer and multimer. Finally, the large molecular weight (>300 kDa) of BsAbs may prevent rapid tumor penetration.

Advances in antibody engineering have made it possible to overcome these restrictions by constructing recombinant

bi-specific antibodies (re-BsAbs) that contain only the single chain fragments of variable regions (scFv) of mAbs, but still produce the same effector responses against tumor cells as whole mAbs do (2, 22, 24-26). The re-BsAbs offer several advantages over intact BsAbs. First, the smaller molecule size (30-50 kDa) allows higher penetration into solid tumor tissues. Second, the HAMA reactions are largely reduced due to the lack of an immunogenic Fc domain of mAb. Third, the process of producing highly purified protein is greatly simplified. Finally, the entire protein production procedure can be done on a commercial scale.

Despite the recent advances in bi-specific antibody technology, structural and functional limitations still remain.

Summary of the Invention

This invention provides a first composition of matter comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety.

This invention also provides a polypeptide comprising the amino acid sequence set forth in Figures 20-1 to 20-15 (SEQ ID NO:2).

This invention also provides a polypeptide comprising the amino acid sequence set forth in Figure 25 (SEQ ID NO:4).

This invention further provides a nucleic acid encoding a polypeptide comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety having a length of at least 16 amino residues.

This invention further provides a host-vector system comprising a host cell transfected with the instant expression vector.

This invention further provides a method for producing a polypeptide comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a linker moiety having a length of at least 16 amino residues, which method comprises (a) culturing the instant host-vector system under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

This invention further provides a second composition of matter comprising (a) the above-described composition and

(b) a cell having on its surface the antigen to which the first antigen-binding moiety specifically binds.

This invention further provides a method for increasing the activity of a CD3+ cell comprising contacting the cell with the instant composition.

This invention further provides a method for treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising administering to the subject (a) an agent known to ameliorate the disorder via contact with the abnormal cell, and (b) the instant composition, wherein the first antigen-binding moiety specifically binds to an antigen present on the agent, and the second antigen-binding moiety specifically binds to an antigen present on the abnormal cell.

This invention further provides a method for treating a subject afflicted with a tumor comprising administering to the subject (a) Interleukin-2 (IL-2), (b) T cells, and (c) the antibody designated E3Bi.

This invention further provides a kit for use in treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising (a) the instant composition, wherein the first antigen-binding moiety specifically binds to an antigen present on an agent known to ameliorate the disorder and the second antigen-binding moiety specifically binds to an antigen present on the abnormal cell, and (b) instructions for use.

This invention further provides a kit for use in treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising (a) the first

instant composition, and (b) the agent known to ameliorate the disorder.

Finally, this invention provides a kit for use in treating a subject afflicted with a tumor comprising (a) Interleukin-2 (IL-2), (b) T cells, (c) the antibody designated E3Bi, and (d) instructions for use.

Brief Description of the Figures

Figure 1

This Figure shows the over-expression of EpCAM on tumor cell surfaces but not on normal epithelium. The EpCAM is over-expressed in MCF-7 breast cancer cells (middle) and colorectal cancer cells (left), but not in HBS-100 normal breast epithelial cells (right). Cells were stained with the GA733.2 mAb.

Figure 2

This Figure illustrates the relationship of a T cell carrying a ch-TCR with and without the hinge spacer (H). When the scFv binds to a specific antigen on the tumor cell surface, the connected T cell signaling chain "Y" initiates the T cell activation that will produce non-MHC-restricted tumor-killing activity.

Figure 3

Day 14 ATCs were used for these experiments. T cell populations from both healthy donors and patients were either not transduced (T) or transduced with the empty retrovirus only (SAM), with the retrovirus carrying the GA733.2-derived ch-TCR (GA), or with GA plus a hinge (GAH). The effector-to-target ratios are 5:1 for panels A and B, or 2.5:1 for panel 3C. ELISAs of IFN-γ and TNF-α were performed after 24 hr incubation. Supernatants (50 µl) were collected for ELISAs in triplicate. The target cell lysis was determined after incubation for 4 hr at 37°C by the ^{51}Cr-release assay. Only data from healthy donors are shown in panel C because there is no different cytotoxicity observed using either patients' or normal donors' ATCs. Panels A and B show that cytokine production was increased by the hinge addition (GAH with

a hinge and GA without). Panel C shows that cytotoxicity was also increased by about two-fold in the GAH group.

Figure 4

The ch-TCR with a hinge (GAHγ-EN) shows greatly increased T cell aggregations with the tumor cells in comparison to the ch-TCR without the hinge (GAγ-EN). These photographs were taken after co-cultivation of tumor cells and T cells for 4 hr at 37°C at an effector-to-target ratio of 2:1. The arrows point at tumor cells, LS174T. "T cell", non-transduced T cells plus tumor cells; "SAM-EN", T cells transduced with expression vector only without the gene of interest; "GAγ-EN", with the hinge; "EN", an internal ribosome entry site in the vector.

Figure 5

The cytotoxicity of ch-TCR-transduced T cells only occurs when they are exposed to EpCAM-positive tumor cells (LS174T) at an E:T ratio of 5:1 for 24 hr at 37°C. The SD is indicated in both panels. These data also demonstrate that there is no significant difference between using the γ- or ζ-chain as the ch-TCR signaling domain to induce the cytolytic function of these transduced T cells.

Figure 6

These photographs show that the BsAb-mediated aggregation of T cells and tumor cells is specific to the mAb used. Day 14 cultured ATCs armed with 50 ng of OKT3/9184 BsAb bind (aggregate) to MCF-7 (upper left). There is no aggregation in three negative controls: ATCs armed with 50 ng of irrelevant BsAb (upper right), unarmed (lower left), or armed with a mixture of 250 ng of non-conjugated OKT3 and 250 ng of non-conjugated 9184 (lower right). The effector-to-target ratio is 25:1. These photos were taken after 24 hr co-incubation of MCF-7

cells with the BsAb armed TC. The mAb 9184 is an anti-Her2/neu mAb.

Figure 7

This Figure demonstrates that as few as 5 ng BsAb per $1x10^6$ T cells can trigger the cytotoxicity mediated by armed T cells. This cytotoxicity assay was performed using MCF-7 cells. The data presented in this Figure are summarized from three experiments in three different donors. This Figure shows composite titration curves for unarmed TCs and ATCs armed with 0.5, 5.0 and 50.0 ng of OKT3/9184 BsAb at effector-to-target ratios of between 5 and 25 to 1; unarmed (▼) or armed with 0.5 (•), 5.0 (■), and 50 (♦) ng BsAb/$1x10^6$ ATCs/ml.

Figure 8

This Figure shows that 40% of mice treated with BsAb-armed ATCs survived. The BsAb, OKT3XT84.66, was used for this experiment. SCID mice received 3Gy of total body irradiation to eliminate NK cells to ensure engraftment of tumor cells. The mice received subcutaneous co-injections of armed or unarmed ATCs ($20x10^6$ ATCs) along with CEA-positive LS174T tumor cells ($1x10^6$ cells) (Winn assay). The control group only received tumor cells and no ATCs. All non-ATC mice died of tumor progression by day 15 with tumor size >22mm. On day 100, 40% of mice that received armed BsAb were still alive, while only 10% were alive in the group that only received un-armed ATCs.

Figure 9

This Figure shows the construction of E3Bi into pG1EN vector. VH-VLe, the scFv of GA733.2; VH-VL3, the ScFv of OKT3; SD, splicing donor; SA, splicing acceptor; His, 6xHis-tag; IRES, an internal ribosome entry site; neo^r, a neomycine phosphotransferase gene.

Figure 10

This is an illustration of the re-BsAb, E3Bi. VL, variable light chain of mAb; VH, variable heavy chain of mAb; H, hinge.

Figure 11

This is an illustration of the recombinant bi-specific antibody, E3Bi, which binds the T cell receptor on a T cell and the tumor associated-antigen EpCAM on a tumor cell. Once this complex is formed, the T cell will be activated by the receptor-E3Bi binding, and will become cytotoxic and kill a tumor cell.

Figure 12

T cell aggregation is dependent on the E3Bi doses. E:T = 10:1, Day 15 ATCs, target = LS174T.

Figure 13

This Figure shows a cytotoxicity assay (^{51}Cr release assay) of E3Bi-armed T cells. Target = LS174T, 16 hr assay.

Figure 14

This Figure shows IFN-γ production induced by different doses of E3Bi.

Figure 15

This Figure shows the cloning of a hinge to the 3'-end of EpCAM scFv.

Figure 16

This Figure shows the construction of OKT3 scFv.

Figure 17

This Figure shows the assembly of E3 to pG1EN.

Figure 18

This Figure shows the replacement of a hinge with GS-linker GGGGSGGGGSGGGGS.

Figure 19

This Figure shows a circular map of pG1EN-EH3.His.

Figures 20-1 to 20-15

The complete DNA sequences of E3Bi and its vector have been confirmed by DNA sequencing analysis. This DNA plasmid is called pG1EN-EH3.His. The completed DNA sequence of 8,078 base pairs (SEQ ID NO:1) and the corresponding amino acid sequence (SEQ ID NO:2) are also shown. The scFv of GA733.2 starts at site 1,388, the hinge starts at site 2,169, and the scFv of OKT3 starts at site 2,358. The 6XHis tag starts at site 3,093.

Figure 21

This Figure shows the *in vivo* anti-tumor response of E3Bi in a tumor xenograft model by tumor growth delay. SCID-Beige mice bearing LS174T xenografts were treated intratumoral (IT) injections IL-2 (n=6), or IL-2/ATC (n=8), or IL-2/ATC/E3Bi (n=6) beginning when tumor volumes of mice reached approximately 0.5 cc. Tumor growth delay is reported as the mean number of days (±SD) for tumor volumes of mice from each treatment group to reach 2 cc.

p = 0.0034 is the probability by Kruskal-Wallis non-parametric analysis that tumor growth delay is the same for all treatment groups. p < 0.01 is the probability by

Dunn's multiple comparison analysis that treatment with IL-2/ATC/E3Bi produces the same tumor growth delay in mice as treatment with IL-2 alone; $p > 0.05$ for IL-2/ATC alone.

Figure 22

This Figure shows the survival of LS174T cells from LS174T tumor xenografts excised from SCID-Beige mice 24 h after mice received treatment with: IL-2 (300 IU/injection i.t.) alone; IL-2 and ATC (7×10^7 cells/injection i.t.); or IL-2/ATC and low (1 mg/kg i.v.) or high dose (10 mg/kg i.v.) E3Bi. After excision, tumor cells were processed into single-cell suspensions and seeded into cultures in four concentrations with five replicates each. Cells were counted after 7 days. Results are represented as the mean (±SE) surviving fraction of cells from each treatment group compared to the IL-2 treatment group. $p < 0.001$, IL-2 or IL-2/ATC vs. IL-2/ATC/E3Bi (10 mg/kg); $p < 0.001$, IL-2/ATC vs. IL-2/ATC/E3Bi (1 mg/kg); $p < 0.05$, IL-2/ATC/E3Bi (1 mg/kg) vs. IL-2/ATC/E3Bi (10 mg/kg).

Figure 23

This Figure shows that E3Bi significantly triggers the cytotoxicity of PBMC ($p = 0.0088$). 1, 2, and 3 day cytotoxicity assays (CML) were conducted on PBMC as the effectors and LS174T colon tumor cells as target cells. The E/T ratio is 5. 100 pmole E3Bi/10^6 effectors were used. The error bars show the standard deviations from the triplicate. This Figure also shows that there was some non-MHC restricted and non-specific cytolytic activity of T cells in E3Bi- group; however, this cytolytic activity is insignificant, $p > 0.05$.

Figure 24

The cDNA sequence of E3Bi (SEQ ID NO:3).

Figure 25

The protein sequence of E3Bi (SEQ ID NO:4).

Figures 26-1 to 26-5

Alternative protein sequence version of pG1EN-EH3.His (SEQ ID NO:5). The completed DNA sequence of 8,078 base pairs (SEQ ID NO:1) is also shown.

Detailed Description of the Invention

Definitions

As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below.

"Activated T Cell," also referred to herein as "ATC," shall have the meaning normally ascribed to it in the art. Characteristics of ATC include, without limitation, resumption of cell cycle, elevated IL-2 secretion, upregulated IL-2 receptor expression, limited proliferation, and differentiation into effector cells.

"Administering" shall mean delivering in a manner which is effected or performed using any of the various methods and delivery systems known to those skilled in the art. Administering can be performed, for example, intravenously, pericardially, orally, via implant, trans-mucosally, transdermally, intramuscularly, sub-cutaneously, intraperitoneally, intrathecally, intra-lymphatically, intralesionally, or epidurally. Administering can be performed, for example, once, a plurality of times, and/or over one or more extended periods.

The term "antibody" includes, by way of example, both naturally occurring antibodies (e.g., IgG, IgM, IgE and IgA) and non-naturally occurring antibodies. The term "antibody" also includes polyclonal and monoclonal antibodies, and fragments thereof (e.g., antigen-binding portions). Furthermore, the term "antibody" includes chimeric antibodies, wholly synthetic antibodies, human antibodies, humanized antibodies, and fragments thereof.

"BsAb", also referred to herein as "bi-specific antibody", shall include, without limitation, a composition of matter comprising two operably affixed moieties, wherein each moiety is capable of binding to an antigen and comprises an antibody. BsAbs include, for example, (i) compositions comprising whole antibodies tethered together, (ii) single antibodies having two antigen-binding domains, each specific for a different antigen, (iii) single chain polypeptides, each comprising two antigen-binding domains linked via a region of at least 16 amino acid residues, and (iv) compositions comprising antigen-binding portions of antibodies operably affixed via chemical linkers.

"E3Bi" in this application is equivalent to "E3-Bi" found in the priority application.

"Flexible linker moiety" shall mean any chemical or biochemical moiety which (i) joins two antigen-binding moieties, (ii) comprises at least one chemical bond about which rotation is permitted, and (iii) permits the unhindered binding of each antigen-binding moiety joined thereto to its respective antigen. In the preferred embodiment, the flexible linker moiety permits binding of the two antigen-binding moieties to their respective antigens located on different cells (e.g., permitting the first antigen-binding moiety to bind to its antigen on a tumor cell, and the second antigen-binding moiety to bind to its antigen on a T cell).

"Host cells" include, but are not limited to, bacterial cells, yeast cells, fungal cells, insect cells, and mammalian cells. Mammalian cells can be transfected by methods well-known in the art such as calcium phosphate

precipitation, electroporation and microinjection.

"Mammalian cell" shall mean any mammalian cell. Mammalian cells include, without limitation, cells which are normal, abnormal and transformed, and are exemplified by neurons, epithelial cells, muscle cells, blood cells, immune cells, stem cells, osteocytes, endothelial cells and blast cells.

"Non-activated T cell" shall have the meaning normally ascribed to it in the art. Characteristics of a non-activated T cell include, without limitation, quiescence of cell cycle, non-proliferation and non-differentiation.

The terms "nucleic acid", "polynucleotide" and "nucleic acid sequence" are used interchangeably herein, and each refers to a polymer of deoxyribonucleotides and/or ribonucleotides. The deoxyribonucleotides and ribonucleotides can be naturally occurring or synthetic analogues thereof.

"Pharmaceutically acceptable carriers" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1 M and preferably 0.05 M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers can be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions and suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Intravenous vehicles include

fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases, and the like.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein, and each means a polymer of amino acid residues. The amino acid residues can be naturally occurring or chemical analogues thereof. Polypeptides, peptides and proteins can also include modifications such as glycosylation, lipid attachment, sulfation, hydroxylation, and ADP-ribosylation.

"Specifically bind" shall mean that, with respect to the binding of an antigen-binding moiety to its respective antigen, the moiety binds to that antigen with a greater affinity than that with which it binds to most or all other antigens. In the preferred embodiment, the moiety binds to that antigen with a greater affinity than that with which it binds to all other antigens.

"Stem cell" shall mean, without limitation, a cell that gives rise to a lineage of progeny cells. Examples of stem cells include CD34+ cells and embryonic stem cells. Surface adhesion molecules present on stem cells include, without limitation, IL-3 receptor, IL-6 receptor, IL-11 receptor, c-kit, VLA-4, VLA-5, L-selectin, PECAM-1 and Beta-1 integrin.

"Subject" shall mean any animal, such as a mammal or a bird, including, without limitation, a cow, a horse, a sheep, a pig, a dog, a cat, a rodent such as a mouse or rat, a chicken, a turkey and a primate. In the preferred embodiment, the subject is a human being.

"Vector" shall mean any nucleic acid vector known in the art. Such vectors include, but are not limited to, plasmid vectors, cosmid vectors, and bacteriophage vectors.

Embodiments of the Invention

This invention provides a first composition of matter comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety.

The flexible linker moiety can comprise, for example, a polymer or a polypeptide. In one embodiment, the polypeptide has a length of at least 16 amino acid residues. In another embodiment, the polypeptide has a length of between 16 amino acid residues and about 100 amino acid residues. In another embodiment, the polypeptide has a length of between 50 amino acid residues and about 75 amino acid residues. In a further embodiment, the polypeptide has a length of about 63 amino acid residues, and/or comprises all or a portion of an antibody hinge region (e.g., CD8α Ig hinge-like region). Preferably, the polypeptide has the amino acid sequence encoded by nucleotides 2170-2358 shown in Figures 20-1 to 20-15 (SEQ NO ID:1).

Preferably, in the first composition, the first and second antigen-binding moieties specifically bind to different antigens. In one embodiment, the first antigen-binding moiety specifically binds to a tumor cell surface antigen. In another embodiment, the first antigen-binding moiety specifically binds to a cell surface antigen such as CD2, CD3, CD56 or other T cell or

NK cell surface antigen. In a further embodiment, the first antigen-binding moiety specifically binds to a tumor cell surface antigen, and the second antigen-binding moiety specifically binds to a CD3+ cell surface antigen. In the preferred embodiment, the tumor cell surface antigen is EpCAM, and the CD3+ cell surface antigen is CD3. Other antigens include, for example, the breast cancer-associated antigen HER2. Antibodies against this antigen are known.

In another embodiment, the first antigen-binding moiety comprises the antigen-binding portion of an anti-EpCAM antibody, and the second antigen-binding moiety comprises the antigen-binding portion of the antibody designated OKT3. In another embodiment, the anti-EpCAM antibody comprises the antigen-binding portion of the antibody designated GA733.2.

In the first composition, each antigen-binding moiety preferably comprises the antigen-binding portion of an antibody. The antigen-binding portions can be, for example, Fab portions.

In one embodiment of the first composition, the composition comprises a single polypeptide chain which forms the first and second antigen-binding moieties and the linker moiety. In another embodiment, each of the first and second antigen-binding moieties further comprises a second polypeptide chain.

This invention further provides a polypeptide comprising the amino acid sequence set forth in Figures 20-1 to 20-15 (SEQ ID NO:2). This polypeptide is referred to herein as E3Bi, and comprises an anti-EpCAM and anti-CD3 domain.

This invention further provides a polypeptide comprising the amino acid sequence set forth in Figure 25 (SEQ ID NO:4).

This invention further provides a nucleic acid encoding a polypeptide comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety having a length of at least 16 amino residues. In one embodiment, the nucleic acid has the nucleotide sequence shown in Figures 20-1 to 20-15 (SEQ ID NO:1). In another embodiment, the nucleic acid has the nucleotide sequence shown in Figure 24 (SEQ ID NO:3).

The nucleic acid can be, for example, DNA or RNA, and is preferably DNA. In another embodiment, the nucleic acid is an expression vector. Expression vectors include, for example, plasmids, cosmids, bacteriophages and eukaryotic viruses. In one embodiment, the eukaryotic virus is an adenovirus or a retrovirus.

This invention further provides a host-vector system comprising a host cell transfected with the instant expression vector.

This invention further provides a method for producing a polypeptide comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety having a length of at least 16 amino residues, which method comprises (a) culturing the instant host-vector system under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

This invention further provides a second composition of matter comprising (a) the instant composition and (b) a cell having on its surface the antigen to which the first antigen-binding moiety specifically binds. In one embodiment, the cell is a CD3+ cell and the first antigen-binding moiety specifically binds to CD3.

In another embodiment, the cell is a T cell, the first antigen-binding moiety comprises the antigen-binding portion of the antibody designated OKT3, and the second antigen-binding moiety comprises the antigen-binding portion of the antibody designated GA733.2. In one embodiment, the composition of (a) is present in a ratio of from about 5-500 ng per million cells of (b).

This invention further provides a method for increasing the activity of a CD3+ cell comprising contacting the cell with the first composition.

This invention further provides a method for treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising administering to the subject (a) an agent known to ameliorate the disorder via contact with the abnormal cell, and (b) the above-described composition, wherein the first antigen-binding moiety specifically binds to an antigen present on the agent, and the second antigen-binding moiety specifically binds to an antigen present on the abnormal cell.

In one embodiment, the subject is selected from the group consisting of a cow, a horse, a sheep, a pig, a dog, a cat, a rabbit and a primate. In the preferred embodiment, the subject is a human.

The disorder treated by the instant method can be any

disorder mediated by an abnormal cell. Such disorders include, without limitation, cancer and specifically tumors. Cancer includes, without limitation, solid tumors, metastatic tumor cells and nonsolid cancers of the blood, marrow, and lymphatic systems. Tumors include, for example, carcinomas (cancers derived from epithelial cells), sarcomas (derived from mesenchymal tissues), lymphomas (solid tumors of lymphoid tissues), and leukemias (marrow or blood borne tumors of lymphocytes or other hematopoietic cells).

In a particular embodiment of the instant method, the agent is a CD3+ cell, the first antigen-binding moiety specifically binds to CD3 (or any other T cell antigen), and the second antigen-binding moiety specifically binds to EpCAM. In another embodiment, the composition comprises the polypeptide whose amino acid sequence is shown in Figures 20-1 to 20-15 (SEQ ID NO:2). In another embodiment, the composition comprises the polypeptide whose amino acid sequence is shown in Figure 25 (SEQ ID NO:4).

This invention further provides a method for treating a subject afflicted with a tumor comprising administering to the subject (a) Interleukin-2 (IL-2), (b) T cells, and (c) the antibody designated E3Bi. The T cells can be, for example, activated T cells or non-activated T cells.

In one embodiment, the subject is selected from the group consisting of a cow, a horse, a sheep, a pig, a dog, a cat, a rabbit and a primate. In the preferred embodiment, the subject is a human.

This invention further provides a kit for use in treating a subject afflicted with a disorder mediated by the

presence of an abnormal cell, comprising (a) the first instant composition, wherein the first antigen-binding moiety specifically binds to an antigen present on an agent known to ameliorate the disorder and the second antigen-binding moiety specifically binds to an antigen present on the abnormal cells, and (b) instructions for use.

This invention further provides a kit for use in treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising (a) the first instant composition, and (b) the agent known to ameliorate the disorder. In one embodiment of the instant kits, the composition of (a) comprises a polypeptide having the sequence shown in Figures 20-1 to 20-15 (SEQ ID NO:2). In another embodiment of the instant kits, the composition of (a) comprises a polypeptide having the sequence shown in Figure 25 (SEQ ID NO:4).

Finally, this invention provides a kit for use in treating a subject afflicted with a tumor comprising (a) Interleukin-2 (IL-2), (b) T cells, (c) the antibody designated E3Bi, and (d) instructions for use. The T cells can be, for example, activated T cells or non-activated T cells.

This invention will be better understood from the Experimental Details that follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

Experimental Details

Introduction

The immunotherapeutic approach of using armed T cells with chemically conjugated bi-specific monoclonal antibodies (BsAbs) has shown specific cytotoxity against tumor cells. This BsAb carries dual specific "arms", one arm recognizing and specifically binding to a tumor associated antigen (TAA), the other one to the CD3 receptor of T cells. When a BsAb bridges a T cell and a tumor cell, the armed T cell can bypass the major histocompatibility complex (MHC) restriction and become a TAA-specific cytotoxic T lymphocyte (CTL). In the treatment of cancers, BsAbs have shown improvement of survival in humans and complete tumor eradication in animals.

Unfortunately, the use of these BsAbs is limited for long-term treatments for the following reasons. (1) Patients develop immune reactions against the BsAb because the BsAb was originally generated in mice. (2) The BsAb production is inconsistent from batch to batch. Using antibody engineering technologies, a genetically engineered recombinant BsAb (E3Bi) was constructed which contains only the sites for tumor and T cell binding but not the immunogenic site of the antibodies which causes unwanted reactions in patients. Generating highly purified protein products is greatly simplified and the entire procedure can be used in commercial production.

The TAA that E3Bi targets is called EpCAM (epithelial cell adhesion molecule). EpCAM is over-expressed in all adenocarcinomas. Since EpCAM is a membrane protein and there is no soluble form in the serum to block antigen

binding sites, and since EpCAM over-expressed in nearly all types of tumors, EpCAM was chosen as an ideal target for the E3Bi approach.

A re-BsAb was constructed from the mAb GA733.2 and mAb OKT3, and called E3Bi. GA733.2 recognizes EpCAM (8).

<u>The tumor targets</u>

EpCAM (epithelial cell adhesion molecule, also called EGP-2, EGP-40, 17-1A, KSA) is a TAA that is over-expressed in all adenocarcinomas (23). Since EpCAM is a membrane protein and there is no soluble form of it in the serum to block antigen binding sites, EpCAM is an ideal target for the re-BsAb approach. Figure 1 shows the surface antibody staining of EpCAM in colorectal (left) and breast (middle) cancer, as well as in normal epithelium (right).

EpCAM is a well-studied and characterized tumor antigen. Two antibodies, CO17-1A and GA733.2, bind to EpCAM, but at different epitopes and with different affinities. CO17-1A has been used in clinical trials to treat colorectal cancer following surgery (6). However, there were no detectable immune responses reported. To direct a T cell to specifically target a TAA, the T cell receptor (TCR) can be engineered so that it carries the binding sites of a mAb that recognizes a TAA. This technique is also called a "T-body" or chimeric TCR (ch-TCR) approach. Daly *et al.* showed that only GA733.2 ch-TCR, not CO17-1A ch-TCR, bound to EpCAM-positive tumor targets (4), probably because GA733.2 has a greater affinity than does CO17-1A ($5x10^8$ M^{-1} compared with $0.7x10^8$ M^{-1}, respectively (8)).

Preliminary Work

Addition of a hinge spacer can significantly increase the tumor-binding and killing function of a ch-TCR

Two ch-TCRs (Figure 2) have been constructed. One has a hinge (H) insert and the other does not. Both ch-TCRs contain the scFv of the mAb GA733.2 that binds to the EpCAM and a T cell signaling domain that triggers T cell activation. Both the FcR γ-chain (GAHγ) and TCR ζ-chain (GAHζ) were used as the T cell signaling domain. This ch-TCR was transduced into an activated T cell (ATC) via a retrovirus. These results show that T cells carrying this ch-TCR specifically and efficiently target and lyse tumor cells (18), and the hinge spacer can increase the specific tumor lytic function (Figures 3 and 4).

The addition of a hinge between the scFv and γ-chain greatly increased cytotoxic activity (18) (Figure 3). These results support the belief that the hinge spacer between these two scFv motifs improves binding efficiency to the targets as well as cytotoxicity.

Matthias Mack's group has designed a re-BsAb against EpCAM that is generated from a M79 hybridism (anti-17-1A) and they have shown its specific cytotoxicity *in vitro* (10, 14, 15). A 5-amino acid linker (G_4S_1) bridges these two scFvs in their respective re-BsAb. To date, they have not reported any results from *in vivo* experiments or clinical trials. However, their work has contributed to an improved design of re-BsAb: (1) the efficacy of the dual-headed recombinant antibody which contains only the scFv domains; (2) the feasibility of using mammalian CHO cells to express the fully functional recombinant protein; (3) the unnecessary addition of a co-stimulation

portion in a re-BsAb construct; and (4) the stability of re-BsAb at 4°C for at least 6 months.

The mAb, GA733.2, specifically binds to EpCAM-positive tumor cells, and not to EpCAM-negative cells

The cell line NCI-H716 is originally generated from cecum tumor cells that are EpCAM-negative. Using H716 as negative control, it was demonstrated that GA733.2 only targets EpCAM-positive cells (Figure 5).

BsAbs are effective at specifically targeting tumor cells and generating cytolytic activity, and pre-arming T cells with BsAb before infusion increases the efficiency of BsAb-mediated tumor killing

To evaluate the potential efficacy of the E3Bi approach in future cancer therapy, the chemically heteroconjugated BsAb (OKT3/9184) targeting Her2/neu-positive breast cancer MCF-7 cells (21) was tested. In these experiments, there was a significant difference observed between adding the BsAb directly to the T cell and tumor cell mixture and pre-arming by adding the BsAb to T cells first, and then adding the armed T cells to the tumor targets (21) (data not shown). Figure 6 shows, in the upper left panel, that activated T cells (ATCs) which had been cultured for 14 days and armed with 50 ng of BsAb (per 10^6 cells) bind to and then kill MCF-7 cells. The lower left panel shows the un-armed ATC control. The ATCs in the upper right panel have been armed with irrelevant mAbs and those shown in the lower right have been armed with non-conjugated mAbs.

In order to determine the optimal arming dose for OKT3/9184, dose titration studies were performed at effector-to-target ratios of from 5:1 to 25:1. Increasing the arming doses led to increasing the mean percentage

specific cytotoxicity. Figure 7 shows the specific cytolytic activity at different doses using ATCs from three healthy donors.

Using SCID mice and Winn assays (co-injection of tumor and T cells), a BsAb (OKT3xT84.66) was tested that specifically targets CEA (carcinoembryonic antigen)-positive colon cancer. The CEA-positive colorectal tumor cell line, LS174T, was used for these studies. Figure 8 shows that OKT3xT84.66 BsAb can prevent tumor progression and death in 40% of the mice.

<u>Further Experiments</u>

It is maintained that (1) the re-BsAb E3Bi, derived from mAb GA733.2, binds to EpCAM on tumor cells better than the re-BsAb from mAb CO17-1A; (2) a hinge addition between two scFv motifs enhances the binding efficiency; and (3) pre-arming ATCs with the re-BsAb before infusion improves efficiency and minimizes clinical toxicity.

<u>Purpose and Methods of Study</u>

Specific Aims

To test this position, the following experiments were designed as set forth below.

Experiment 1: Construct E3Bi from two single chain fragments of variable regions (scFvs) of mAbs GA733.2 and OKT3, and insert a linker from the CD8α hinge-like region (H) between these two scFvs. As a control, the H linker is replaced with a traditional glycine-serine linker, $(G_3S_1)_3$. A 6xHis-tag is also constructed into the C-terminus of this recombinant protein for affinity

purification purposes.

Experiment 2: Express E3Bi in the mammalian cell line CHO and affinity purify E3Bi.

Experiment 3: Evaluate the specific cytolytic function of E3Bi *in vitro* (using EpCAM-positive colon cancer cell line LS174T, and using EpCAM-negative cecum cancer cell line H716 as a negative control) and *in vivo* (using Beige-SCID mice).

Significance of the Instant Technology

The biggest challenge for cancer treatment is to direct a patient's own immune system to fight cancer. In general, tumor growth is the result of a defective immune system in which the MHC (major histocompatibility complex) fails to present tumor antigens to the immune system and to generate enough specific cytotoxic T lymphocytes (CTL). Therefore, the adoptive immunotherapy strategies hold promise for cancer therapy because the focus of these treatments is to redirect a patient's own immune system to bypass MHC-restricted recognition and directly target tumor cells.

There are three major approaches in recently developed adoptive immunotherapy protocols. (1) Genetic modification of T cells to carry a chimeric T cell receptor (ch-TCR) that can recognize a specific tumor cell. Upon binding to the tumor cell, the ch-TCR will trigger the T cell to become a CTL and kill specific tumor cells. Because of the involvement of retrovirus production and gene transduction into ATCs *in vitro*, this treatment could be very expensive. (2) Dendritic cell (DC)-mediated tumor vaccination. Tumor antigens are

introduced into DCs so that they can present these tumor antigens to T cells and generate specific CTL. This strategy has not yet shown clinical success. Because there is no product that can be manufactured and specially trained medical technicians and facilities are required to perform this procedure, this treatment could also be very expensive and inconsistent. (3) Use of a conjugated bi-specific antibody (BsAb) molecule as a bridge between a tumor cell and a T cell so that the tumor cell will directly trigger the T cell to become a tumor-specific CTL.

Although the ch-TCR and DC approaches are important regarding proof-of-principle, they are very difficult, inconsistent and expensive to use in treating patients. Among these three approaches, the BsAb approach holds the greatest promise for clinical applications. It is technically feasible and straightforward.

The engineered recombinant BsAb approach (re-BsAb) overcomes the limitations of chemically heteroconjugated BsAbs because only the binding sites of the antibodies are selectively engineered, and not the regions that may cause side effects such as HAMA reactions. The re-BsAb product can be pure and consistent from lot to lot, while the chemically conjugated BsAb is only about 15-30% pure and the product is very inconsistent. The other advantage of this re-BsAb is that large-scale production is possible.

Again, this invention provides a re-BsAb with improved tumor-killing efficiency. This is accomplished in several ways: (1) using an antibody that has higher binding affinity; (2) adding a longer spacer between two binding sites; (3) producing this protein in mammalian

cells; and (4) arming a patient's T cells with this re-BsAb *in vitro* before infusing the patient.

Relevance to Cancer

Relapse rates remain unacceptably high after conventional treatments currently in use for solid tumors, like adjuvant chemotherapy/radiotherapy or even stem cell transplantation. There is an urgent need for nontoxic and tumor-specific approaches following both conventional and high dose chemotherapy to eradicate residual tumor cells and improve overall and disease-free survival. The goal of the E3Bi approach is to redirect a patient's own immune system to specifically eradicate residual tumor cells following conventional treatments.

Because arming T cells with E3Bi will turn every T cell into a tumor-antigen specific CTL, E3Bi offers a very effective cancer immunotherapy approach. This product will have much less toxicity because the patient's own T cells will be stimulated to eradicate tumor cells. Pre-arming T cells before infusion will further increase the efficiency and specificity of this re-BsAb. Multiple infusions of these armed T cells over a longer period of time are expected to eradicate residual tumor cells more effectively compared to other immunotherapy approaches. The specificity of E3Bi is unique because these armed T cells will locally deposit at a specific tumor site and kill tumor cells. Furthermore, they will also attack residual tumor cells that have already spread prior to surgery.

Because colorectal cancer has the highest incidence among all types of cancer in the U.S., patients with colorectal cancer are envisioned as an important treatment group.

Since EpCAM, the cell surface tumor marker recognized by E3Bi, is over-expressed in all adenocarcinomas (23), a very important aspect of E3Bi is that it has use with respect to most solid tumors as well.

It is expected that this re-BsAb will not only eradicate residual tumor cells, but will be part of adjuvant therapy for a variety of EpCAM+ tumors.

Features of E3Bi

The design of E3Bi is unique and offers several advantages over other re-BsAbs that have been published (25).

(i) The vector pG1EN is used for production of re-BsAb for the first time. Based on previous experience, this vector is highly effective in penetrating mammalian cell membranes, integrating cDNA into the host genome and promoting gene expression.

(ii) Mammalian cells (CHO cells) are used as E3Bi producer cells because mammalian proteins produced in the traditional bacterial cell *E. coli* may not fold properly and therefore may not function correctly.

(iii) The hinge spacer (63 amino acids) used for E3Bi has never been used for re-BsAb construction. The longer linker between two scFvs in E3Bi will provide the space needed for the interaction of a tumor cell and a T cell (18) and, therefore, is expected to increase the binding and tumor killing efficiency of E3Bi.

(iv) mAb GA733.2 is used for constructing a re-BsAb for the first time. Both GA733.2 and CO17-1A target EpCAM,

but at two different epitopes (7). GA733.2 has a higher affinity for EpCAM antigen than does CO17-1A and produces stronger cytotoxicity against EpCAM-positive tumor cells (4). Increasing the affinity for a tumor antigen enhances the cytotoxicity of a bi-specific antibody.

(v) T cells from a patient are activated, expanded, armed with E3Bi and frozen for later infusion into the same patient. This *in vitro* arming protocol is the first of its kind used for a re-BsAb. It is believed that this approach not only provides a large quantity of activated and armed tumor-killing T cells, but also reduces the possible toxicity and increases the efficiency of E3Bi.

Detailed Experimental Methods

(1) Construction of E3Bi cDNA into a high expression vector

Figures 9-11 illustrate the design of E3Bi. (Also shown are the cloning of a hinge to the 3'-end of EpCAM scFv (Figure 15), the construction of OKT3 scFv (Figure 16), the assembly of E3 to pG1EN (Figure 17), the replacement of a hinge with GS-linker GGGGSGGGGSGGGGS (Figure 18), and a circular map of pG1EN-EH3.His (Figure 19)).

The E3Bi cDNA is generated by combining variable light (V_L) and heavy (V_H) chains of mAbs GA733.2 and OKT3 that are amplified by PCR. The E3Bi cDNA is then inserted into an expression vector, pG1EN. PG1EN is generated from the Maloney murine leukemia virus (MMLV) and is replication incompetent due to the lack of three genes that are essential for virus formation, *gag, env* and *pol*. This insures against retroviral replication. Based on previous experience (18), this vector is highly efficient in producing stably transduced mammalian cells and promoting

gene expression. The anti-CD3 scFv is generated from OKT3 hybridoma cells (ATCC, Rockville, MD).

The E3Bi gene expression is driven by long terminal repeats (LTR). This vector contains a leader sequence from the k light chain to penetrate cell membranes, a neomycin phosphotransferase gene (neo^r) for drug selection, a splicing donor (SD)/splicing acceptor (SA) to enhance the efficiency of transcription, and an internal ribosome entry site (IRES) for driving neo^r gene transcription. A 6xHis-tag is added to the C-terminal end for affinity purification of this re-BsAb protein. These two scFvs are linked through a long hinge that is cloned from the CD8α hinge-like region. By adding distance from the scFv to the plasma membrane, the hinge spacer has shown increased tumor binding and killing activity in connection with the chimeric TCR approach (16, 18). Figure 3 shows that in both healthy donors and patients, the ch-TCR with a hinge (GAH) significantly increased the specific tumor cytotoxicity and cytokine secretion (IFN-γ and TNF-α) by about two-fold (compared to a ch-TCR without a hinge) (18). However, the hinge approach has never before been applied for re-BsAb construction. The hinge is expected to give the re-BsAb flexibility and rotational freedom leading to a better bridge between a tumor cell and a T cell.

(2) Expression of E3Bi in eukaryotic cells

Most re-BsAbs are expressed in a traditional prokaryotic expression system (24). However, the re-BsAb protein may not fold properly in prokaryotic cells (14). Therefore, a eukaryotic cell line, the Chinese hamster ovary cell line (CHO, GIBCO Life-technologies, Rockville, MD), is transfected. Specifically, CHO is transfected with the

standard $CaPO_4$ precipitation method (17) and cultured in the presence of the selection drug, G418. The stably transfected CHO cells form colonies after 10-14 days. The colonies are selected for the highest quantity of the re-BsAb production and evaluated by ELISA for the presence of a 6xHis-tag (Ni-NTA HisSorb Plates, QIAGEN, Valencia, CA). The re-BsAb is secreted into the culture medium that is used directly for functional evaluation without further purification. The CHO clone with the highest yield of re-BsAb is grown as non-adherent cells in a serum-free medium specially constituted for CHO (CD-CHO, GIBCO). The medium containing E3Bi is collected every 24 hr or as otherwise determined.

(3) Functional assays of E3Bi

(3.1) In vitro studies

Specific cytolytic and cytokine production assays are performed in both EpCAM-positive (LS174T from ATCC) and negative cells (H716 from ATCC) using the same techniques as described before (18, 21). Figure 8 demonstrates that, using anti-EpCAM mAb (GA733.2) staining, LS174T colorectal cells show very strong surface EpCAM expression.

For these *in vitro* studies, T cells from healthy donors are isolated from 40 cc peripheral blood, activated with anti-CD3 mAb at 10-50 ng/$1x10^6$ T cells/ml, and expanded for 14 days in the presence of 100 IU of IL-2 and 10% fetal calf serum in the medium, RPMI-1640 (BioWittaker, Walkersville, MD). On day 14, the ATCs are armed with different doses of E3Bi and rocked for 1 hr at 4°C. The cells are washed twice with RPMI-1640 to eliminate excess unbound E3Bi. The armed and unarmed ATCs are added to the

target tumor cells at effector-to-target ratios from 1:1 to 10:1. Cytotoxicity assays (^{51}Cr release assay) and IFN-γ production assays (ELISA) are performed in triplicate. The dose, time and temperature in the arming procedure are evaluated. To test the specific targeting of E3Bi against EpCAM, a blocking assay is performed using the anti-Id antibody against the scFv of GA733.2. The cytotoxicity and ELISA assays are analyzed statistically with a standard statistical package, a paired *t*-test or Wilcoxon signed tank test using the SigmaStat. All *in vitro* assays are repeated with at least 5 unrelated subjects. The significant cytotoxic functions of E3Bi are analyzed with a paired *t*-test or Wilcoxon signed tank test using SigmaStat.

(3.2) In vivo studies

In vivo functional assays are performed in animal models. Four to eight week-old female beige SCID mice are used for these studies (Taconic Pharm, Germantown, NY). These mice carry the SCID mutation that causes a deficiency of both T and B cells resulting in cytotoxic T cell and macrophage defects as well as selective impairment of NK cell function. The animals are maintained in accordance with NIH animal care guidelines.

(3.2.1) Winn assay

The mice are divided into two groups; one group with "Winn Assay", which means $1x10^6$ tumor cells are co-injected with armed ATCs (dose range from $1x10^6$ to $10x10^6$) subcutaneously into the upper right thigh of each animal or with unarmed T cells as a control. Tumor development is documented weekly. The other group is injected only with $1x10^6$ tumor cells subcutaneously into the upper right

thigh of each animal. Once the tumor is established (>5mm, about 4 weeks), armed or unarmed T cells at different doses are injected twice a week directly into the center of the tumor mass. As a control, mice from both groups are divided into three sub-groups: the first group receives no T cells; the second group is injected with unarmed T cells, and the third group is injected with armed T cells with E3Bi. The tumor development is measured and documented every 2 days. The tumor cells used for these *in vivo* studies are LS174T (human colorectal adenocarcinoma cells). T cells are extracted from the peripheral blood of both healthy donors and patients. The animals are sacrificed by CO_2 gas overdose once the tumor size exceeds 1.5 cm. By week 8-10 after treatment, all animals are sacrificed. All data are analyzed using a paired *t*-test or Wilcox test on signed tank test using SigmaStat.

(3.2.2) Tumor xenograft model – Xenografted mice with EpCAM+ tumor cells

The *in vivo* anti-tumor response of E3Bi was also evaluated in a tumor xenograft model by tumor growth delay assay. In SCID-Beige mice bearing xenografted LS174T tumors, the average time for tumors to reach four times their pre-treatment volume (0.5 cc) varied significantly between the following three treatment groups ($p = 0.0034$): animals treated by intratumoral (i.t.) injections with IL-2 alone; with IL-2 plus ATC; and with IL-2 plus E3Bi/ATC. Administration of ATC with IL-2 resulted in a tumor growth delay of 7 days compared to IL-2 treatment alone ($p > 0.05$), while addition of E3Bi to the treatment regimen significantly increased tumor growth delay by 12 days compared to IL-2 alone. ($p < 0.01$).

As shown in Figure 21, these results show that E3Bi significantly prolongs the survival rate of tumor-bearing mice, and therefore, provide a therapeutic advantage for using E3Bi with ATC/IL-2 to increase tumor growth inhibitions.

The same xenografted mouse model was also used to evaluate the trafficking and high does tolerance of parenterally-administered E3Bi *in vivo*. Four-week old SCID-Beige mice were divided into four groups: i.t. injection of IL-2 only ($1x10^4$ IU/kg); i.t. injection of IL-2 and ATC ($2x10^9$ cells/kg); i.v. injection of a low (1 mg/kg) or high (10 mg/kg) dose E3Bi along with an i.t. injection of IL-2/ATC. Each mouse received two i.v. injections (day 1 and day 3) of E3Bi and two i.t. injections of IL-2/ATC, day 1 with 14-day old ATC from a healthy donor (N4) and day 3 with 17-day old. Tumor necrosis was observed within 48 h after the injection in mice receiving high dose E3Bi, but not in mice receiving low dose E3Bi, ATC/IL-2 or IL-2 only. High dose E3Bi was well-tolerated with no evidence of any side effects.

The tumor size more than doubled in the mice receiving only ATC/IL-2 while it remained largely unchanged in mice receiving low dose E3Bi after 7 days from the first injection. In addition to the observed necrosis (E) of tumors in mice receiving high dose of E3Bi, the tumors in these mice demonstrate partial regression within 7 days of initial treatment.

Figure 22 further supports the targeting specificity of E3Bi to EpCAM+ over-expressing tumors *in vivo*. Mice with established LS174T tumors were treated with ATC or ATC followed by an IV injection of low or high does E3Bi, and excised 24 h later. The viability of treated cells was

measured as the surviving fraction of tumor cells after *in vivo* treatment with IL-2, IL-2/ATC and IL-2/ATC/E3Bi. Though ATC treatment alone produced no cytotoxic effect on tumor cells, administration of low dose (1 mg/kg) E3Bi in conjunction with ATC treatment produced a 40% decrease in tumor cell survival. Increasing the E3Bi dose to 10 mg/kg significantly decreased the tumor cell survival by 90% ($p < 0.05$). Combined with the tumor growth inhibition studies, these results show that E3Bi delivered systematically traffics, binds and produces cytotoxic effects to EpCAM+ over-expressing tumor cells *in vivo*.

(3.2.3) E3Bi triggered cytotoxicity of non-activated T cell activation

E3Bi also directly triggers non-activating T-cells to kill tumor cells. For example, E3Bi triggered CD4+ and CD8+ populations in peripheral blood mononuclear cells (PBMC) to become activated in the presence of LS174T tumor cells (data not shown). Both T cell activation markers, CD25 and CD69, increased upon activation by E3Bi and resulted in increased cytolytic activity of T-cells, as shown in Figure 23.

Figure 23 illustrates that E3Bi triggers cytotoxicity in PMBC, which include non-activated T cells. 1, 2, and 3 day cytotoxicity assays (CML) were conducted using PBMC as the effectors and LS174T colon tumor cells as target cells. On day 3, the cytotoxicity of PBMC rose to 70%, and therefore, shows that E3Bi significantly triggers the cytotoxicity of PBMC ($p = 0.0088$). This Figure also shows some non-MHC restricted and non-specific cytolytic activity of T cells in the E3Bi- group; however, this cytolytic activity is insignificant, $p > 0.05$.

(3.3) Anticipated obstacles

(3.3.1) Clearance of E3Bi by kidney before it can attack tumors

One major concern for a small sized re-BsAb is that it can be cleared rapidly by the kidney, and therefore, the amount of its retention by the tumor is very limited (3, 22). To overcome this problem, T cells are pre-armed *in vitro* with E3Bi before infusion so that the small E3Bi will remain attached to the CD3 receptor on the T cells while traveling in the body and, therefore, be protected from rapid kidney clearance. More importantly, pre-arming the T cells *in vitro* will dramatically improve the killing efficiency (data not shown). Existing methodology enables one to pre-arm T cells *in vitro* for future clinical trials. The pre-arming procedure includes (1) mixing day 14 ATCs with different doses of E3Bi in a tube and rocking for one hour at 4°C; (2) washing twice to remove unbound E3Bi; and (3) infusing the armed T cells at a concentration of $1x10^7$ cells/ml.

(3.3.2) No costimulation

Without CD28 costimulation, T cell activation can result in activation-induced T cell apoptosis (AICD) and as a consequence, reduced tumor killing efficiency *in vivo*. These phenomena have not been observed using ch-TCR (18, 19) and BsAb (10). However, to confirm that there is no AICD in re-BsAb-mediated tumor killing activities and the armed ATCs can be recycled *in vivo*, bystander-killing assays, apoptosis assays (Annexin V staining) and ^{3}[H]-thymidine proliferation assays are performed. Following tumor exposure, the fate of armed T cells is studied with and without the E3Bi.

(4) Affinity purification of E3Bi

The high producer cells are grown in suspension in the serum-free medium, CHO-S-SFM II (GIBCO), which is a constituted medium developed specifically for CHO cells growing in suspension. The supernatant containing the released E3Bi is collected every 24 hr and affinity purified by applying it to Ni-NTA spin columns. These columns can purify up to 150 mg of E3Bi in a one-step affinity purification of 6xHis-tag-containing recombinant protein (QIAGEN). The columns are washed and eluted according to the manufacturer's instructions. The quality of the purified product is evaluated by denaturing gel electrophoresis and Western blot. For the short term, E3Bi is stored in phosphate-buffered saline at 4°C, lyophilized and stored at -20°C for the long term.

(5) Affinity purification of E3Bi

Collected supernatant containing the E3Bi is applied to a Ni-NTA agarose column (nickel-charged resin, QIAGEN). The concentration of eluted E3Bi is tested with the BCA testing kit (BCA-200 Protein Assay Kit, Bio-Rad, Hercules, CA). The final product is filtered through a 0.22 mm filter, aliquoted in 1 mg protein/ml PBS/vial and stored in the -20°C freezer. This affinity purification is conducted in a cold box (4°C) in the GMP lab.

(6) Activation and expansion of T cells in vitro

It is routine to activate T cells in gas-permeable plastic bags with anti-CD3 antibody, OKT3 (OrthoBiotech, Raritan, NJ). Briefly, T cells from healthy donors or patients are transferred into bags at a concentration of 1×10^6 $CD3^+$ cells/ml RPMI culture medium (BioWittaker)

supplemented with 2-5% human serum, 100-500 IU of IL-2/ml and 20 ng OKT3/ml. T cells are maintained at a concentration of $1x10^6$ cells/ml for 14 days.

(7) Arming activated T cells with E3Bi

The procedure for arming T cells with E3Bi is adopted from established procedures for using chemically heteroconjugated BsAb. Briefly, day 14 ATCs are transferred into a tube, washed and re-suspended in an optimal volume of culture medium containing the optimized dose of E3Bi. After incubation, excess E3Bi is washed twice by centrifugations. The armed ATCs are either aliquoted and frozen or directly used for functional studies.

(8) E3Bi-Mediated T Cell Killing

As shown in Figure 12, T cell aggregation is dependent on the E3Bi doses. Specifically, three photos show the binding of T cells (small round dots) and tumor cells (growing in "island-like" groups) mediated by E3Bi. The CHO cell culture supernatant that contains E3Bi was added to the T cell and tumor cell mixture. Panel A contains no CHO supernatant and there is no binding or aggregation between T cells and tumor cells. In panel B (12.5%), there are a significant number of T cells attached to the tumor cells. In panel C (25%), all tumor cells are aggregated with T cells. These panels clearly show that E3Bi can direct T cells to kill tumor cells. The concentration of E3Bi in the supernatant was not determined. As a control, the same CHO supernatant that contains recombinant protein other than E3Bi did not produce the same aggregation effects (data not shown).

Figure 13 shows a ^{51}Cr release assay of E3Bi-armed T cells. This cytotoxicity assay shows the percentage of targets (tumor cells) that are killed by the effectors (T cells) in the presence of E3Bi. "E/T" indicates the number of T cells per tumor cell. These data show that at 16 hours, about 70% of tumor cells are killed at E/T = 10, and 50% at E/T = 5. Supernatant collected from 50% confluent E3Bi-transduced CHO cell culture was used for this assay. The "mock" is a control, wherein only an "empty vector" (i.e., without an E3Bi insert) was transduced into CHO cells and the supernatant was used.

As shown in Figure 14, IFN-γ production is induced by different doses of E3Bi. CHO cell culture supernatant containing secreted E3Bi was added to T cell and tumor cell mixtures at different doses in microliters as indicated. The absolute concentration of E3Bi was not determined. The cytotoxic function of T cells is usually indicated by the amount of their IFN-γ production. These data clearly show that E3Bi induces significant IFN-γ production in a dose-dependent manner, while the control group does not stimulate IFN-γ production.

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What is claimed is:

1. A composition of matter comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety.

2. The composition of claim 1, wherein the flexible linker moiety comprises a polymer.

3. The composition of claim 1, wherein the flexible linker moiety comprises a polypeptide.

4. The composition of claim 3, wherein the polypeptide has a length of at least 16 amino acid residues.

5. The composition of claim 4, wherein the polypeptide has a length of between 16 amino acid residues and about 100 amino acid residues.

6. The composition of claim 5, wherein the polypeptide has a length of between 50 amino acid residues and about 75 amino acid residues.

7. The composition of claim 6, wherein the polypeptide has a length of about 63 amino acid residues.

8. The composition of claim 7, wherein the polypeptide comprises the amino acid sequence encoded by nucleotide 2170-2358 shown in Figures 20-1 to 20-15 (SEQ ID NO:1).

9. The composition of claim 3, wherein the polypeptide comprises all or a portion of an antibody hinge

region.

10. The composition of claim 1, wherein the first and second antigen-binding moieties specifically bind to different antigens.

11. The composition of claim 10, wherein the first antigen-binding moiety specifically binds to a tumor cell surface antigen.

12. The composition of claim 10, wherein the first antigen-binding moiety specifically binds to a CD3+ cell surface antigen.

13. The composition of claim 10, wherein the first antigen-binding moiety specifically binds to a tumor cell surface antigen, and the second antigen-binding moiety specifically binds to a CD3+ cell surface antigen.

14. The composition of claim 13, wherein the tumor cell surface antigen is EpCAM, and the CD3+ cell surface antigen is CD3.

15. The composition of claim 14, wherein the first antigen-binding moiety comprises the antigen-binding portion of an anti-EpCAM antibody, and the second antigen-binding moiety comprises the antigen-binding portion of the antibody designated OKT3.

16. The composition of claim 15, wherein the anti-EpCAM antibody comprises the antigen-binding portion of the antibody designated GA733.2.

17. A polypeptide comprising the amino acid sequence set

forth in Figures 20-1 to 20-15 (SEQ ID NO:2).

18. A polypeptide comprising the amino acid sequence set forth in Figure 25 (SEQ ID NO:4).

19. The composition of claim 1, wherein each antigen-binding moiety comprises the antigen-binding portion of an antibody.

20. The composition of claim 19, wherein each antigen-binding portion of the antibody is a Fab portion.

21. The composition of claim 19, wherein the antibody is chimeric.

22. The composition of claim 3, wherein the composition comprises a single polypeptide chain which forms the first and second antigen-binding moieties and the linker moiety.

23. The composition of claim 22, wherein each of the first and second antigen-binding moieties further comprises a second polypeptide chain.

24. A nucleic acid encoding a polypeptide comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety having a length of at least 16 amino residues.

25. The nucleic acid of claim 24 having the nucleotide sequence shown in Figures 20-1 to 20-15 (SEQ ID NO:1).

26. The nucleic acid of claim 24 having the nucleotide

sequence shown in Figure 24 (SEQ ID NO:3).

27. The nucleic acid of claim 24, wherein the nucleic acid is DNA or RNA.

28. The nucleic acid of claim 27, wherein the nucleic acid is DNA.

29. The nucleic acid of claim 24, wherein the nucleic acid is an expression vector.

30. The nucleic acid of claim 29, wherein the expression vector is selected from the group consisting of a plasmid, a cosmid, a bacteriophage and a eukaryotic virus.

31. The nucleic acid of claim 30, wherein the eukaryotic virus is an adenovirus or a retrovirus.

32. A host-vector system comprising a host cell transfected with the expression vector of claim 29.

33. A method for producing a polypeptide comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety having a length of at least 16 amino residues, which method comprises (a) culturing the host-vector system of claim 32 under conditions permitting the expression of the polypeptide, and (b) recovering the polypeptide so expressed.

34. A composition of matter comprising (a) the composition of claim 1 and (b) a cell having on its surface the antigen to which the first antigen-

binding moiety specifically binds.

35. The composition of claim 34, wherein the cell is a CD3+ cell and the first antigen-binding moiety specifically binds to CD3.

36. The composition of claim 35, wherein the cell is a T-cell, the first antigen-binding moiety comprises the antigen-binding portion of the antibody designated OKT3, and the second antigen-binding moiety comprises the antigen-binding portion of the antibody designated GA733.2.

37. The composition of claim 34, wherein the composition of (a) is present in a ratio of from about 5-500 ng per million cells of (b).

38. A method for increasing the activity of a CD3+ cell comprising contacting the cell with the composition of claim 1.

39. A method for treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising administering to the subject (a) an agent known to ameliorate the disorder via contact with the abnormal cell, and (b) the composition of claim 1, wherein the first antigen-binding moiety specifically binds to an antigen present on the agent, and the second antigen-binding moiety specifically binds to an antigen present on the abnormal cell.

40. The method of claim 39, wherein the subject is selected from the group consisting of a cow, a horse, a sheep, a pig, a dog, a cat, a rabbit and a

primate.

41. The method of claim 40, wherein the subject is a human.

42. The method of claim 39, wherein the disorder is a tumor.

43. The method of claim 42, wherein the agent is a CD3+ cell, the first antigen-binding moiety specifically binds to CD3, and the second antigen-binding moiety specifically binds to EpCAM.

44. The method of claim 39, wherein the composition comprises the polypeptide whose amino acid sequence is shown in Figures 20-1 to 20-15 (SEQ ID NO:2).

45. The method of claim 39, wherein the composition comprises the polypeptide whose amino acid sequence is shown in Figure 25 (SEQ ID NO:4).

46. A method for treating a subject afflicted with a tumor comprising administering to the subject (a) Interleukin-2 (IL-2), (b) T cells, and (c) the antibody designated E3Bi.

47. The method of claim 46, wherein the T cells are activated T cells.

48. The method of claim 46, wherein the T cells are non-activated T cells.

49. The method of claim 46, wherein the subject is selected from the group consisting of a cow, a horse, a sheep, a pig, a dog, a cat, a rabbit and a

primate.

50. The method of claim 49, wherein the subject is a human.

51. A kit for use in treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising (a) the composition of claim 1, wherein the first antigen-binding moiety specifically binds to an antigen present on an agent known to ameliorate the disorder and the second antigen-binding moiety specifically binds to an antigen present on the abnormal cell, and (b) instructions for use.

52. A kit for use in treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, comprising (a) the composition of claim 1, and (b) the agent known to ameliorate the disorder.

53. The kit of claim 51 or 52, wherein the composition of (a) comprises a polypeptide having the sequence shown in Figures 20-1 to 20-15 (SEQ ID NO:2).

54. The kit of claim 51 or 52, wherein the composition of (a) comprises a polypeptide having the sequence shown in Figure 25 (SEQ ID NO:4).

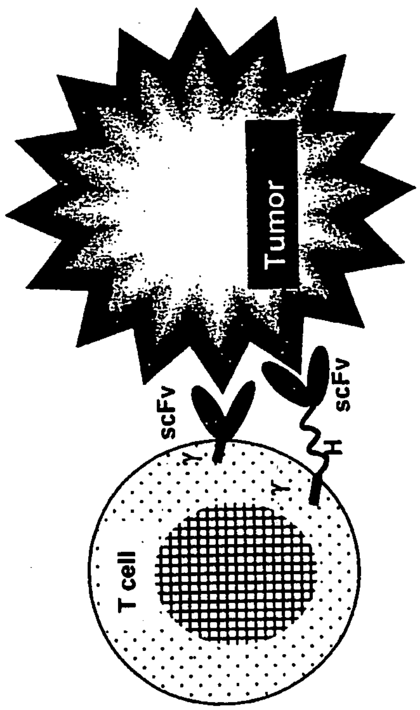
55. A kit for use in treating a subject afflicted with a tumor comprising (a) Interleukin-2 (IL-2), (b) T cells, (c) the antibody designated E3Bi, and (d) instructions for use.

56. The kit of claim 55, wherein the T cells are activated T cells.

57. The kit of claim 55, wherein the T cells are non-activated T cells.

FIGURE 1

FIGURE 2



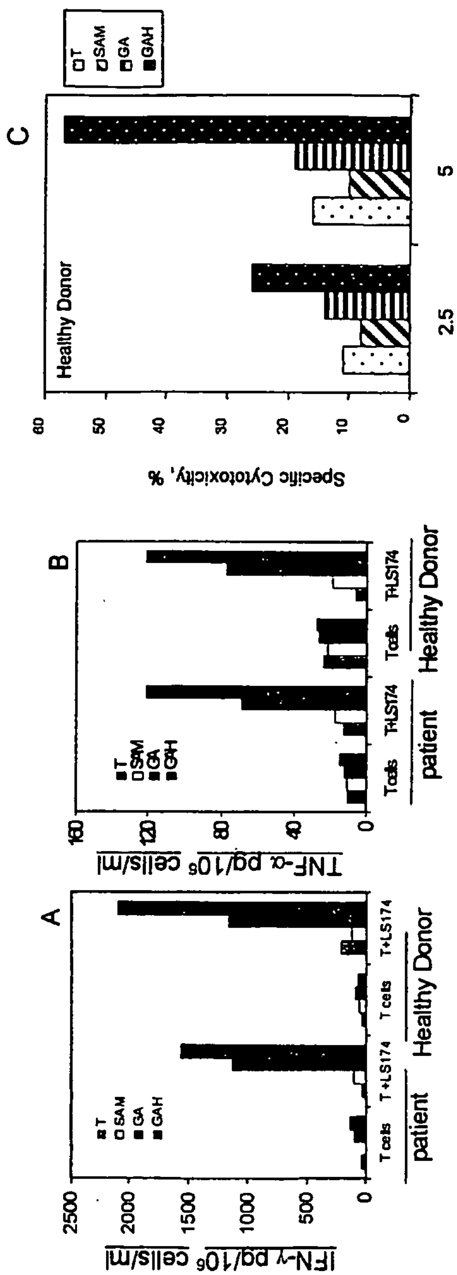

A
IFN-γ pg/10⁶ cells/ml
2500
2000
1500
1000
500
0
T
SAM
GA
GAH
T cells
T+LS174
patient
Healthy Donor
B
TNF-α, pg/10⁶ cells/ml
160
120
80
40
0
C
Healthy Donor
Specific Cytotoxicity, %
60
50
40
30
20
10
0
2.5
5
E:T

FIGURE 3

FIGURE 4

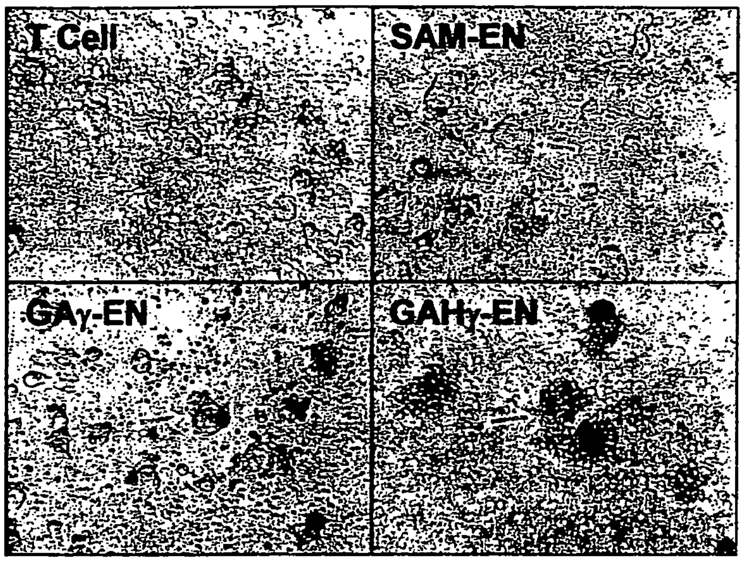


FIGURE 5

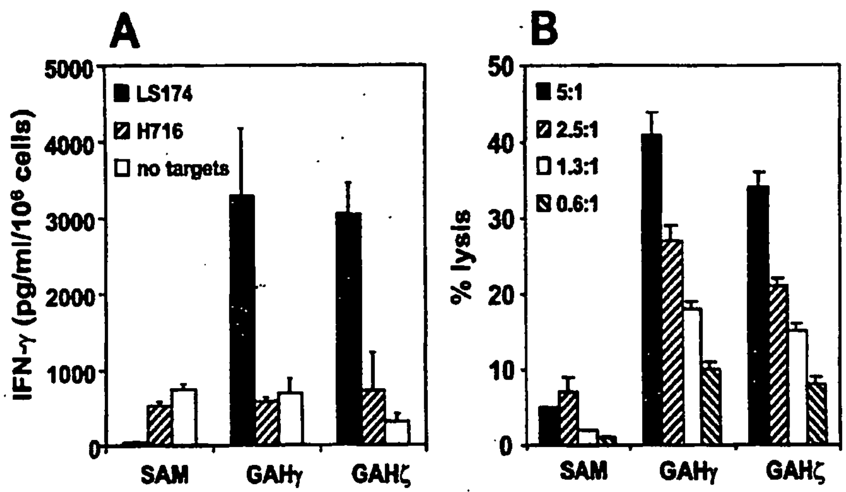

A
IFN-γ (pg/ml/10⁶ cells)
5000
4000
3000
2000
1000
0
LS174
H716
no targets
SAM
GAHγ
GAHζ
B
% lysis
50
40
30
20
10
0
5:1
2.5:1
1.3:1
0.6:1
SAM
GAHγ
GAHζ

FIGURE 6

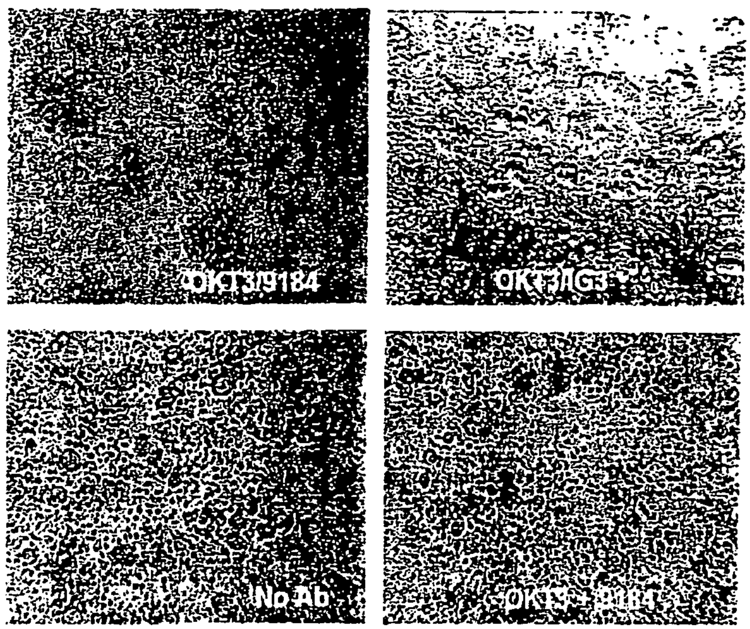


FIGURE 7

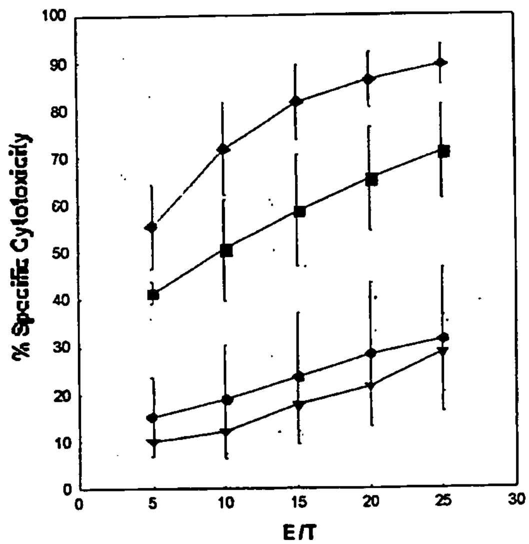
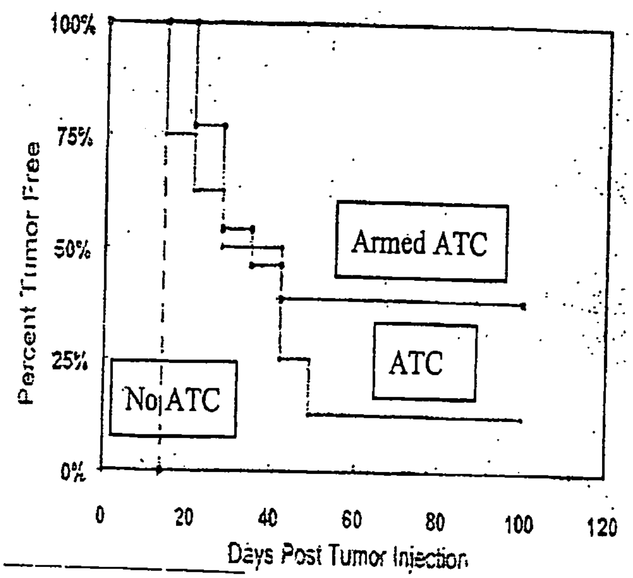
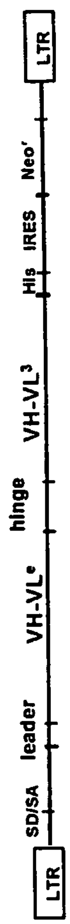

100
90
80
70
60
50
40
30
20
10
0
% Specific Cytotoxicity
0
5
10
15
20
25
30
E/T

FIGURE 8



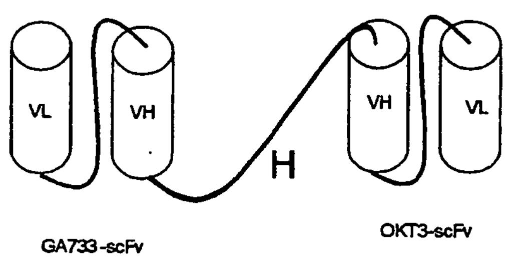
100%
75%
50%
25%
0%
Percent Tumor Free
Armed ATC
ATC
No ATC
0
20
40
60
80
100
120
Days Post Tumor Injection

FIGURE 9



PG1EN-E3.His (pE3.His)

FIGURE 10



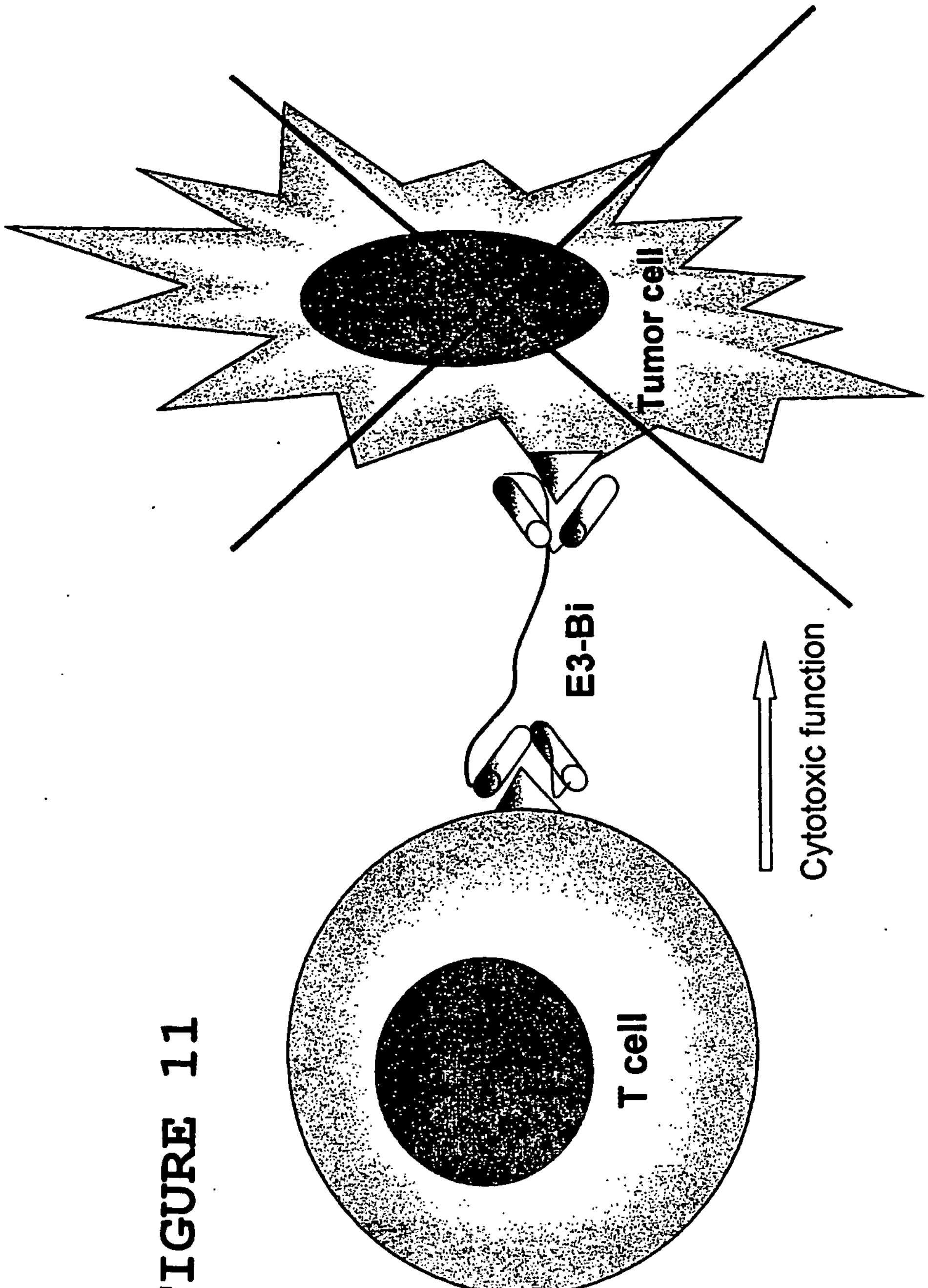


FIGURE 11

FIGURE 12

The T cell aggregation is dependent on the E3-Bi doses
E:T=10:1, Day 15 ATC, target=LS174T

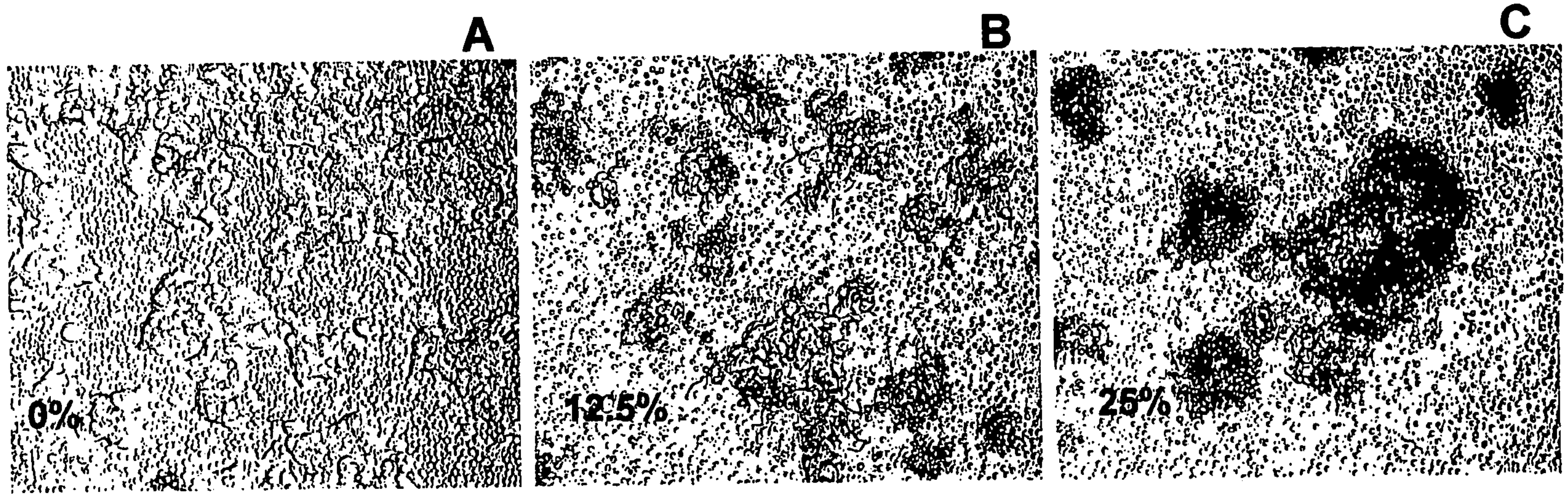
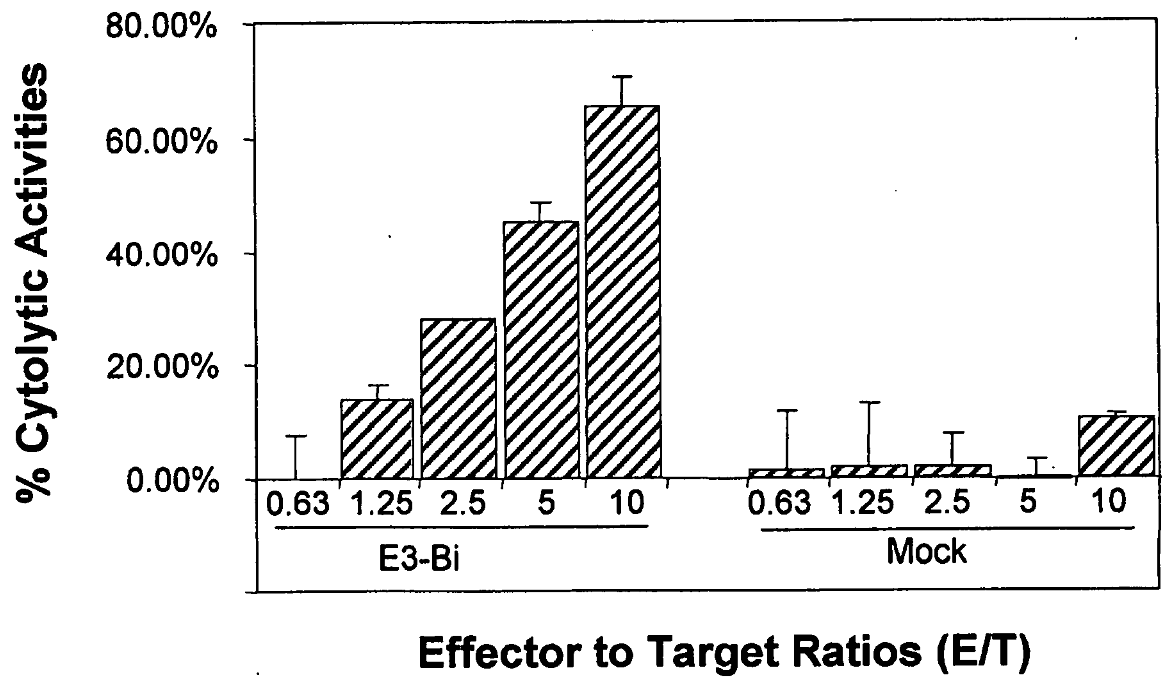


FIGURE 13 **Cytotoxicity Assay (^{51}Cr release assay) of E3-Bi Armed T cells Target = LS174T, 16 hr assay**



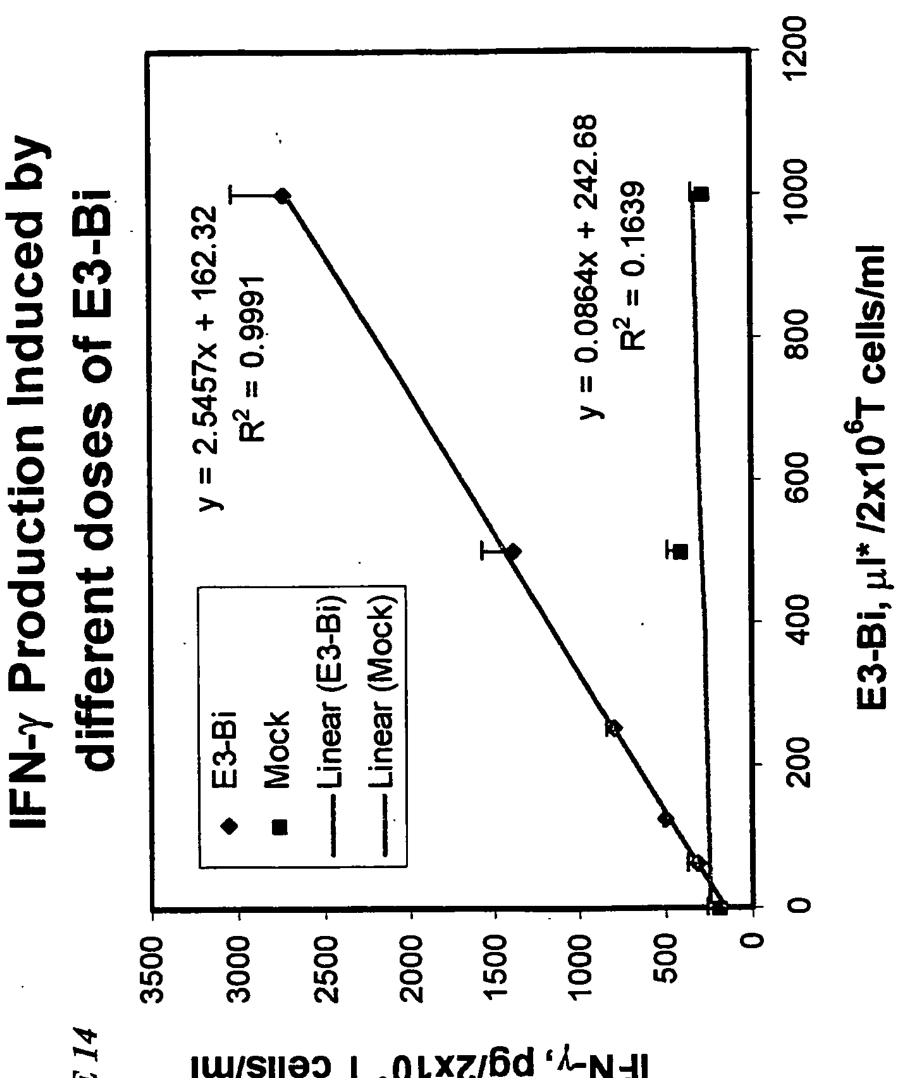

IFN-γ Production Induced by different doses of E3-Bi
E3-Bi
Mock
Linear (E3-Bi)
Linear (Mock)
y = 2.5457x + 162.32
R² = 0.9991
y = 0.0864x + 242.68
R² = 0.1639
IFN-γ, pg/2x10⁶ T cells/ml
E3-Bi, µl* /2x10⁶T cells/ml
0
500
1000
1500
2000
2500
3000
3500
0
200
400
600
800
1000
1200

FIGURE 14

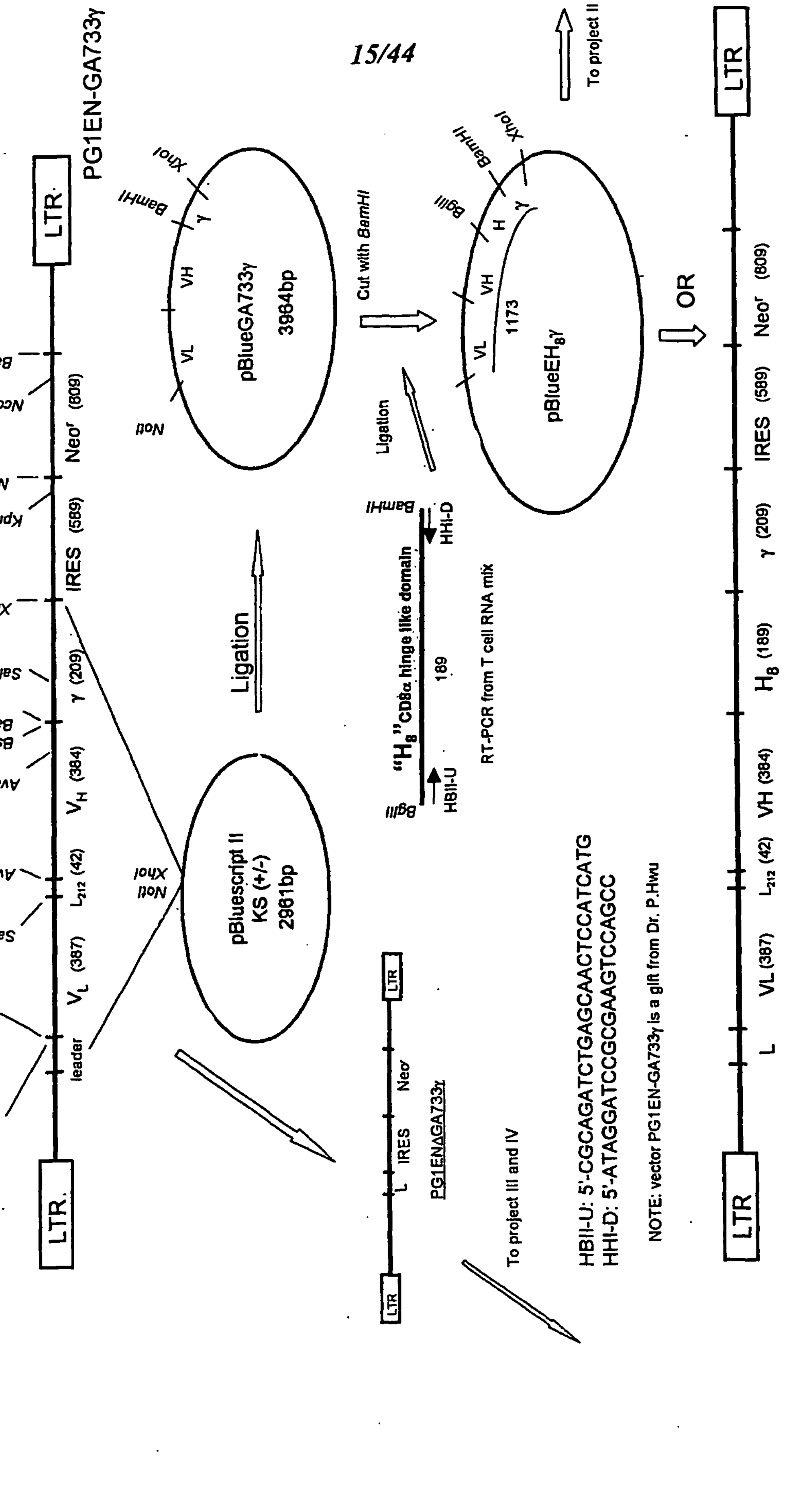
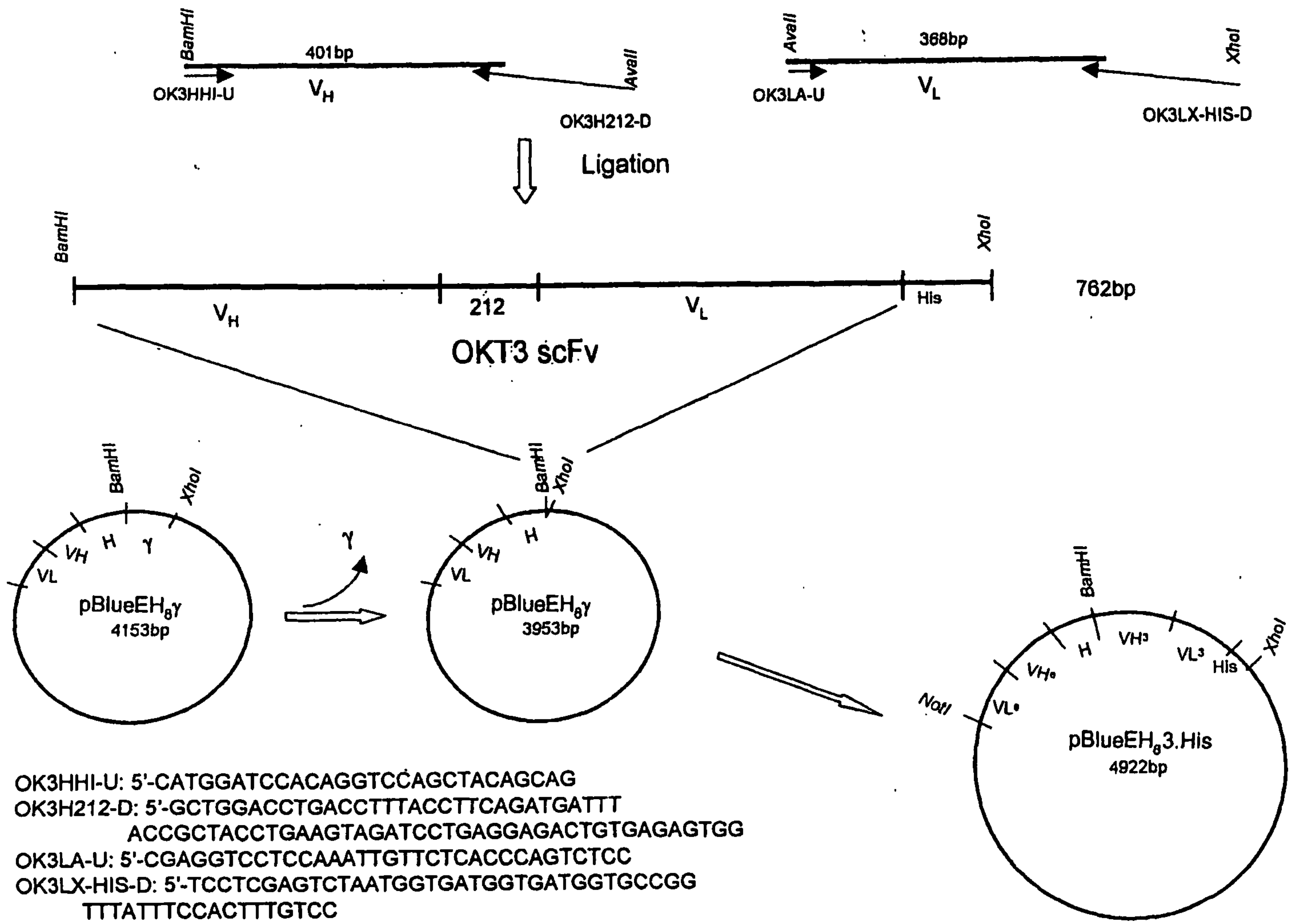

PG1EN-GA733γ
LTR
leader
HpaI SnaBI NotI NcoI XbaI
V_L (387)
SalI
L_{212} (42)
AvaII
V_H (384)
AvaII BstEII BamHI
γ (209)
SalI
XhoI
IRES (589)
KpnI
NcoI
Neo^r (809)
NcoI
BamHI
LTR
pBluescript II KS (+/-) 2961bp
NotI XhoI
Ligation
pBlueGA733γ 3964bp
NotI VL VH γ BamHI XhoI
Cut with BamHI
"H_8" CD8α hinge like domain
BglII HBII-U 189 HHI-D BamHI
RT-PCR from T cell RNA mix
Ligation
pBlueEH$_8$γ
VL VH H γ 1173 BglII BamHI XhoI
To project II
OR
LTR L IRES Neo^r LTR
PG1ENΔGA733γ
To project III and IV
HBII-U: 5'-CGCAGATCTGAGCAACTCCATCATG
HHI-D: 5'-ATAGGATCCGCGAAGTCCAGCC
NOTE: vector PG1EN-GA733γ is a gift from Dr. P.Hwu
LTR L VL (387) L_{212} (42) VH (384) H_8 (189) γ (209) IRES (589) Neo^r (809) LTR

FIGURE 15

FIGURE 16



OK3HHI-U: 5'-CATGGATCCACAGGTCCAGCTACAGCAG
OK3H212-D: 5'-GCTGGACCTGACCTTTACCTTCAGATGATTT
ACCGCTACCTGAAGTAGATCCTGAGGAGACTGTGAGAGTGG
OK3LA-U: 5'-CGAGGTCCTCCAAATTGTTCTCACCCAGTCTCC
OK3LX-HIS-D: 5'-TCCTCGAGTCTAATGGTGATGGTGATGGTGCCGG
TTTATTTCCACTTTGTCC

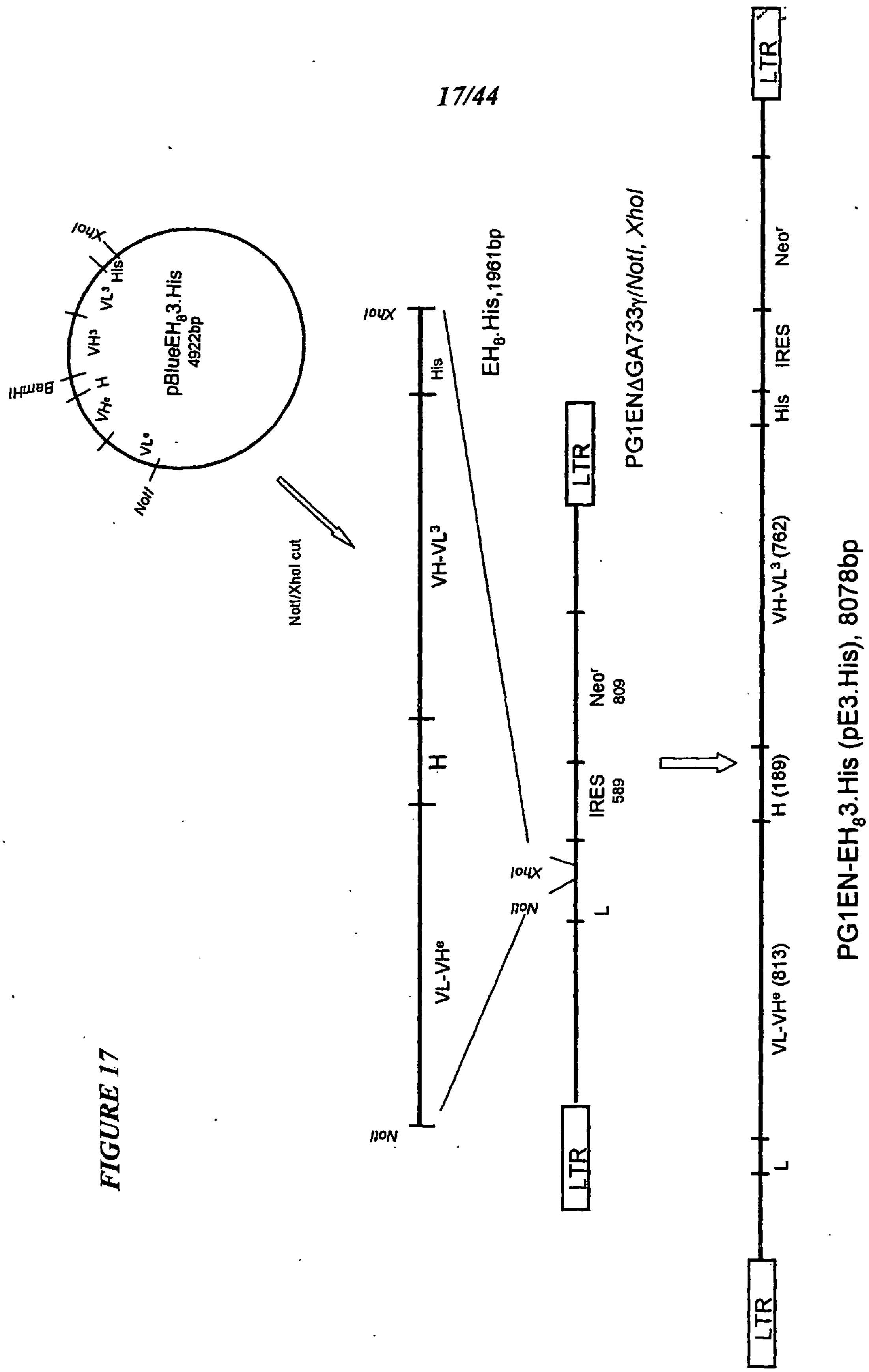
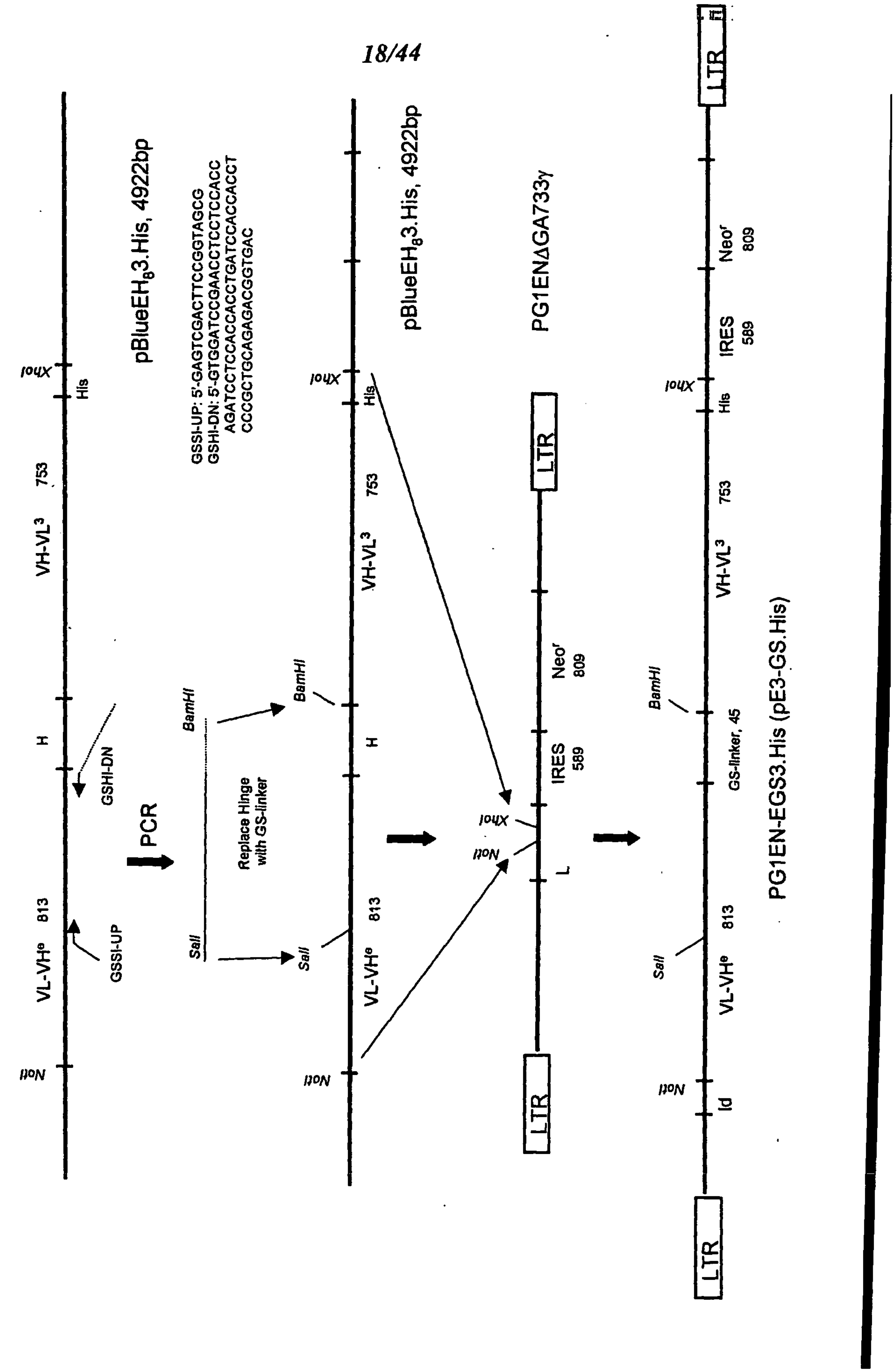

pBlueEH$_8$3.His
4922bp
XhoI
His
VL^3
VH^3
BamHI
H
VH^e
VL^e
NotI
NotI/XhoI cut
EH$_8$.His,1961bp
XhoI
His
VH-VL^3
H
VL-VH^e
NotI
PG1ENΔGA733γ/NotI, XhoI
LTR
Neo^r
809
IRES
589
XhoI
NotI
L
LTR
LTR
Neo^r
IRES
His
VH-VL^3 (762)
H (189)
VL-VH^e (813)
L
LTR
PG1EN-EH$_8$3.His (pE3.His), 8078bp

FIGURE 17



NotI
VL-VH^e 813
GSSI-UP
GSHI-DN
H
VH-VL^3 753
His
XhoI
pBlueEH$_8$3.His, 4922bp
PCR
GSSI-UP: 5'-GAGTCGACTTCCGGTAGCG
GSHI-DN: 5'-GTGGATCCGAACCTCCTCCACC
AGATCCTCCACCACCTGATCCACCACCT
CCCGCTGCAGAGACGGTGAC
SalI
BamHI
Replace Hinge with GS-linker
NotI
SalI
VL-VH^e 813
BamHI
H
VH-VL^3 753
His
XhoI
pBlueEH$_8$3.His, 4922bp
NotI
XhoI
LTR
L
IRES 589
Neo^r 809
LTR
PG1ENΔGA733γ
LTR
Id
NotI
SalI
VL-VH^e 813
GS-linker, 45
BamHI
VH-VL^3 753
His
XhoI
IRES 589
Neo^r 809
LTR
PG1EN-EGS3.His (pE3-GS.His)

FIGURE 18

FIGURE 19

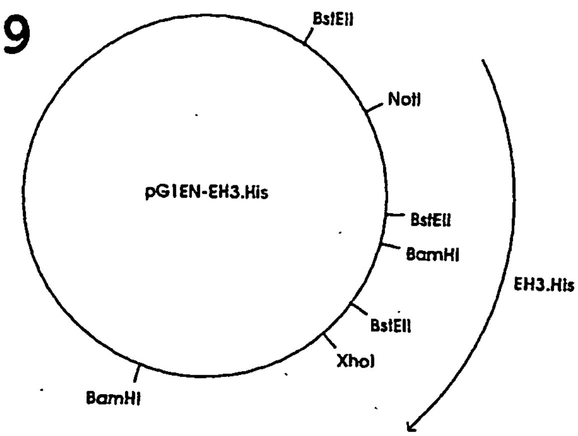

BstEII
NotI
pG1EN-EH3.His
BstEII
BamHI
EH3.His
BstEII
XhoI
BamHI

FIGURE 20-1

```
          9            18            27            36            45            54
AGC CCA CAA CCC CTC ACT CGG CGC GCC AGT CTT CCG ATA GAC TGC GTC GCC CGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   P   Q   P   L   T   R   R   A   S   L   P   I   D   C   V   A   R

         63            72            81            90            99           108
GTA CCC GTA TTC CCA ATA AAG CCT CTT GCT GTT TGC ATC CGA ATC GTG GTC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   P   V   F   P   I   K   P   L   A   V   C   I   R   I   V   V   S

        117           126           135           144           153           162
CTG TTC CTT GGG AGG GTC TCC TCT GAG TGA TTG ACT ACC CAC GAC GGG GGT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   F   L   G   R   V   S   S   E   *   L   T   T   H   D   G   G   L

        171           180           189           198           207           216
TCA TTT GGG GGC TCG TCC GGG ATT TGG AGA CCC CTG CCC AGG GAC CAC CGA CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   F   G   G   S   S   G   I   W   R   P   L   P   R   D   H   R   P

        225           234           243           252           261           270
ACC ACC GGG AGG TAA GCT GGC CAG CAA CCT ATC TGT GTC TGT CCG ATT GTC TAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   T   G   R   *   A   G   Q   Q   P   I   C   V   C   P   I   V   *

        279           288           297           306           315           324
TGT CTA TGT TTG ATG TTA TGC GCC TGC GTC TGT ACT AGT TAG CTA ACT AGC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   L   C   L   M   L   C   A   C   V   C   T   S   *   L   T   S   S

        333           342           351           360           369           378
GTA TCT GGC GGA CCC GTG GTG GAA CTG ACG AGT TCT GAA CAC CCG GCC GCA ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   S   G   G   P   V   V   E   L   T   S   S   E   H   P   A   A   T

        387           396           405           414           423           432
CAG GGA GAC GTC CCA GGG ACT TTG GGG GCC GTT TTT GTG GCC CGA CCT GAG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   G   D   V   P   G   T   L   G   A   V   F   V   A   R   P   E   E

        441           450           459           468           477           486
GGG AGT CGA TGT GGA ATC CGA CCC CGT CAG GAT ATG TGG TTC TGG TAG GAG ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   S   R   C   G   I   R   P   R   Q   D   M   W   F   W   *   E   T

        495           504           513           522           531           540
AGA ACC TAA AAC AGT TCC CGC CTC CGT CTG AAT TTT TGC TTT CGG TTT GGA ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   T   *   N   S   S   R   L   R   L   N   F   C   F   R   F   G   T
```

FIGURE 20-2 21/44

```
        549         558         567         576         585         594
GAA GCC GCG CGT CTT GTC TGC TGC AGC ATC GTT CTG TGC [illegible] [illegible] TGC [illegible] ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   A   A   R   L   V   C   C   S   I   V   L   C   C   L   C   L   T

        603         612         621         630         639         648
GTG TTT CTG TAT TTG TCT GAA AAT TAG GGC CAG ACT GTT ACC ACT CCC TTA AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   F   L   Y   L   S   E   N   *   G   Q   T   V   T   T   P   L   S

        657         666         675         684         693         702
TTG ACC TTA GGT CAC TGG AAA GAT GTC GAG CGG ATC GCT CAC AAC CAG TCG GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   L   G   H   W   K   D   V   E   R   I   A   H   N   Q   S   V

        711         720         729         738         747         756
GAT GTC AAG AAG AGA CGT TGG GTT ACC TTC TGC TCT GCA GAA TGG CCA ACC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   V   K   K   R   R   W   V   T   F   C   S   A   E   W   P   T   F

        765         774         783         792         801         810
AAC GTC GGA TGG CCG CGA GAC GGC ACC TTT AAC CGA GAC CTC ATC ACC CAG GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   V   G   W   P   R   D   G   T   F   N   R   D   L   I   T   Q   V

        819         828         837         846         855         864
AAG ATC AAG GTC TTT TCA CCT GGC CCG CAT GGA CAC CCA GAC CAG GTC CCC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   I   K   V   F   S   P   G   P   H   G   H   P   D   Q   V   P   Y

        873         882         891         900         909         918
ATC GTG ACC TGG GAA GCC TTG GCT TTT GAC CCC CCT CCC TGG GTC AAG CCC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   V   T   W   E   A   L   A   F   D   P   P   P   W   V   K   P   F

        927         936         945         954         963         972
GTA CAC CCT AAG CCT CCG CCT CCT CTT CCT CCA TCC GCC CCG TCT CTC CCC CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   H   P   K   P   P   P   P   L   P   P   S   A   P   S   L   P   L

        981         990         999        1008        1017        1026
GAA CCT CCT CGT TCG ACC CCG CCT CGA TCC TCC CTT TAT CCA GCC CTC ACT CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   P   P   R   S   T   P   P   R   S   S   L   Y   P   A   L   T   P

       1035        1044        1053        1062        1071        1080
TCT CTA GGC GCC GGA ATT CGC GGC CGT GAC AAG AGT TAC TAA CAG CCC CTC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   L   G   A   G   I   R   G   R   D   K   S   Y   *   Q   P   L   S

       1089        1098        1107        1116        1125        1134
CCA AGC TCA CTT ACA GGC TCT CTA CTT AGT CCA GCA CGA AGT CTG GAG ACC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

FIGURE 20-3 22/44

P S S L T G L L S P A R L E T S

1143 1152 1161 1170 1179 1188
GGC GGC AGC CTA CCA AGA ACA ACT GGA CCG ACC GGT GGT ACC TCA CCC TTA CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
G G S L P R T T G P T G G T S P L P

1197 1206 1215 1224 1233 1242
AGT CGG CGA CAC AGT GTG GGT CCG CCG ACA CCA GAC TAA GAA CCT AGA ACC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
S R R H S V G P P T P D * E P R T S

1251 1260 1269 1278 1287 1296
CTG GAA AGG ACC TTA CAC AGT CCT GCA GAC CAC CCC CAC CGC CCT CAA AGT AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
L E R T L H S P A D H P H R P Q S R

1305 1314 1323 1332 1341 1350
CGG CAT CGC AGC TTG GAT ACA CGC CGC CCA CGT GAA GGC TGC CGA CCC CGG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
R H R S L D T R R P R E G C R P R G

1359 1368 1377 1386 1395 1404
TGG ACC ATC TCT AGA CTG ACG CGG CCG CTA CGT ACC ATG GAT TTT CAG GTG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
W T I S R L T R P L R T M D F Q V Q

1413 1422 1431 1440 1449 1458
ATT TTC AGC TTC CTG CTA ATC AGT GCC TCA GTC ATA ATG TCT AGA GGG AGC ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
I F S F L L I S A S V I M S R G S I

1467 1476 1485 1494 1503 1512
GTA ATG ACC CAA TCT CAC AAA TTC ATG TCC ACA TCA GTA GGA GAC AGT GTC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
V M T Q S H K F M S T S V G D S V S

1521 1530 1539 1548 1557 1566
ATC ACC TGC AAG GCC AGT CAG GAT GTG AGT ACT GCT GTA GCC TGG TAT CAA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
I T C K A S Q D V S T A V A W Y Q Q

1575 1584 1593 1602 1611 1620
AAA CCA GGA CAA TCT CCT AAA CTA CTG ATT TAC TCG GCA TCC GAC CGG TAC ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
K P G Q S P K L L I Y S A S D R Y T

1629 1638 1647 1656 1665 1674
GGA GTC CCT GAT CGC TTC ACT GGC AGT GGA TCT GGG ACG GAT TTC ACT TTC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
G V P D R F T G S G S G T D F T F T

1683 1692 1701 1710 1719 1728

FIGURE 20-4


```
ATC AGC AGT GTG CAG GCT [illegible]A GAC CTG GCA GTT TAT TAC [illegible] CAC CAA CAT TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   S   S   V   Q   A   E   D   L   A   V   Y   Y   C   H   Q   H   Y

        1737        1746        1755        1764        1773        1782
ATT ACT CCT CGG ACG TTC GGT GGA GGC ACA AAG CTG GAA ATA AAA GGG TCG ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   T   P   R   T   F   G   G   G   T   K   L   E   I   K   G   S   T

        1791        1800        1809        1818        1827        1836
TCC GGT AGC GGC AAA TCC TCT GAA GGC AAA GGT CAG GTC CAG CTG CAG CAG TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   S   G   K   S   S   E   G   K   G   Q   V   Q   L   Q   Q   S

        1845        1854        1863        1872        1881        1890
GGA GCT GAG GTG ATG AGG CCT GGG GCC TCA GTG AAG ATA TCC TGC AAG GCT ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   A   E   V   M   R   P   G   A   S   V   K   I   S   C   K   A   T

        1899        1908        1917        1926        1935        1944
GGC TAC ACA TTC ACT AGG TAC TAC ATA CAA TGG GGT AAA AAC AGG CCT GGA CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   Y   T   F   T   R   Y   Y   I   Q   W   G   K   N   R   P   G   H

        1953        1962        1971        1980        1989        1998
GGC CTT GAG TGG ATT GGA GAG ATT TTA CCT GGA ACT CTT ACT AAT TAC AAT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   E   W   I   G   E   I   L   P   G   T   L   T   N   Y   N   E

        2007        2016        2025        2034        2043        2052
AAA TTC AAG GGC AAG GCC GCA TTC ACT GCA GAT AGA TCC TCC AAC ACA GCC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   F   K   G   K   A   A   F   T   A   D   R   S   S   N   T   A   Y

        2061        2070        2079        2088        2097        2106
ATG CAA CTC AGC AGC CTT ACA TCT GAG GAC TCT GCC GTC TAT TAC TGT GCA AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   Q   L   S   S   L   T   S   E   D   S   A   V   Y   Y   C   A   R

        2115        2124        2133        2142        2151        2160
GAT GGT CCC TGG TTT GCT TAC TGG GGC CAA GGA ACC CTG GTC ACC GTC TCT GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   G   P   W   F   A   Y   W   G   Q   G   T   L   V   T   V   S   A

        2169        2178        2187        2196        2205        2214
GCG GAT CTG AGC AAC TCC ATC ATG TAC TTC AGC CAC TTC GTG CCG GTC TTC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   D   L   S   N   S   I   M   Y   F   S   H   F   V   P   V   F   L

        2223        2232        2241        2250        2259        2268
CCA GCG AAG CCC ACC ACG ACG CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   A   K   P   T   T   T   P   A   P   R   P   P   T   P   A   P   T
```

FIGURE 20-5


```
        2277           2286           2295           2304           2313           2322
ATC GCG TCG CAG CCC CTG TCC CTG CGC CCA GAG GCG TGC CGG CCA GCG GCG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   S   Q   P   L   S   L   R   P   E   A   C   R   P   A   A   G

        2331           2340           2349           2358           2367           2376
GGC GCA GTC CAC ACG AGG GGG CTG GAC TTC GCG GAT CCA CAG GTC CAG CTA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   A   V   H   T   R   G   L   D   F   A   D   P   Q   V   Q   L   Q

        2385           2394           2403           2412           2421           2430
CAG TCT GGG GCT GAA CTG GCA AGA CCT GGG GCC TCA GTG AAG ATG TCC TGC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   S   G   A   E   L   A   R   P   G   A   S   V   K   M   S   C   K

        2439           2448           2457           2466           2475           2484
GCT TCT GGC TAC ACC TTT ACT AGG TAC ACG ATG CAC TGG GTA AAA CAG AGG CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   S   G   Y   T   F   T   R   Y   T   M   H   W   V   K   Q   R   P

        2493           2502           2511           2520           2529           2538
GGA CAG GGT CTG GAA TGG ATT GGA TAC ATT AAT CCT AGC CGT GGT TAT ACT AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   Q   G   L   E   W   I   G   Y   I   N   P   S   R   G   Y   T   N

        2547           2556           2565           2574           2583           2592
TAC AAT CAG AAG TTC AAG GAC AAG GCC ACA TTG ACT ACA GAC AAA TCC TCC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   N   Q   K   F   K   D   K   A   T   L   T   T   D   K   S   S   S

        2601           2610           2619           2628           2637           2646
ACA GCC TAC ATG CAA CTG AGC AGC CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   A   Y   M   Q   L   S   S   L   T   S   E   D   S   A   V   Y   Y

        2655           2664           2673           2682           2691           2700
TGT GCA AGA TAT TAT GAT GAT CAT TAC TGC CTT GAC TAC TGG GGC CAA GGC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   A   R   Y   Y   D   D   H   Y   C   L   D   Y   W   G   Q   G   T

        2709           2718           2727           2736           2745           2754
ACT CTC ACA GTC TCC TCA GGA TCT ACT TCA GGT AGC GGT AAA TCA TCT GAA GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   L   T   V   S   S   G   S   T   S   G   S   G   K   S   S   E   G

        2763           2772           2781           2790           2799           2808
AAA GGT CAG GTC CAG CAA ATT GTT CTC ACC CAG TCT CCA GCA ATC ATG TCT GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   G   Q   V   Q   Q   I   V   L   T   Q   S   P   A   I   M   S   A

        2817           2826           2835           2844           2853           2862
TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC
```

FIGURE 20-6 25/44

```
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   P   G   E   K   V   T   M   T   C
```

PCT/US03/12772

```
        2871         2880         2889         2898         2907         2916
ATG AAC TGG TAC CAG CAG AAG TCA GGC ACC TCC CCC AAA AGA TGG ATT TAT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   N   W   Y   Q   Q   K   S   G   T   S   P   K   R   W   I   Y   D

        2925         2934         2943         2952         2961         2970
ACA TCC AAA CTG GCT TCT GGA GTC CCT GCT CAC TTC AGG GGC AGT GGG TCT GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   S   K   L   A   S   G   V   P   A   H   F   R   G   S   G   S   G

        2979         2988         2997         3006         3015         3024
ACC TCT TAC TCT CTC ACA ATC AGC GGC ATG GAG GCT GAA GAT GCT GCC ACT TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   S   Y   S   L   T   I   S   G   M   E   A   E   D   A   A   T   Y

        3033         3042         3051         3060         3069         3078
TAC TGC CAG CAG TGG AGT AGT AAC CCA TTC ACG TTC GGC TCG GGG ACA AAG TTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   C   Q   Q   W   S   S   N   P   F   T   F   G   S   G   T   K   L

        3087         3096         3105         3114         3123         3132
GAA ATA AAC CGG CAC CAT CAC CAT CAC CAT TAG ACT CGA GGA TCA ATT CCG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   I   N   R   H   H   H   H   H   H   *   T   R   G   S   I   P   P

        3141         3150         3159         3168         3177         3186
CTC TCC CTC CCC CCC CCC TAA CGT TAC TGG CCG AAG CCG CTT GGA ATA AGG CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   S   L   P   P   P   *   R   Y   W   P   K   P   L   G   I   R   P

        3195         3204         3213         3222         3231         3240
GTG TGC GTT TGT CTA TAT GTT ATT TTC CAC CAT ATT GCC GTC TTT TGG CAA TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   C   V   C   L   Y   V   I   F   H   H   I   A   V   F   W   Q   C

        3249         3258         3267         3276         3285         3294
GAG GGC CCG GAA ACC TGG CCC TGT CTT CTT GAC GAG CAT TCC TAG GGG TCT TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   P   E   T   W   P   C   L   L   D   E   H   S   *   G   S   F

        3303         3312         3321         3330         3339         3348
CCC TCT CGC CAA AGG AAT GCA AGG TCT GTT GAA TGT CGT GAA GGA AGC AGT TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   R   Q   R   N   A   R   S   V   E   C   R   E   G   S   S   S

        3357         3366         3375         3384         3393         3402
TCT GGA AGC TTC TTG AAG ACA AAC AAC GTC TGT AGC GAC CCT TTG CAG GCA GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   S   F   L   K   T   N   N   V   C   S   D   P   L   Q   A   A
```

FIGURE 20-7


```
     3411        3420        3429        3438        3447        3456
GAA CCC CCC ACC TGG CGA CAG GTG CCT CTG CGG CCA AAA GCG ACG TGT ATA AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   P   P   T   W   R   Q   V   P   L   R   P   K   A   T   C   I   R

     3465        3474        3483        3492        3501        3510
TAC ACC TGC AAA GGC GGC ACA ACC CCA GTG CCA CGT TGT GAG TTG GAT AGT TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   T   C   K   G   G   T   T   P   V   P   R   C   E   L   D   S   C

     3519        3528        3537        3546        3555        3564
GGA AAG AGT CAA ATG GCT CTC CTC AAG CGT ATT CAA CAA GGG GCT GAA GGA TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   K   S   Q   M   A   L   L   K   R   I   Q   Q   G   A   E   G   C

     3573        3582        3591        3600        3609        3618
CCA GAA GGT ACC CCA TTG TAT GGG ATC TGA TCT GGG GCC TCG GTG CAC ATG CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   E   G   T   P   L   Y   G   I   *   S   G   A   S   V   H   M   L

     3627        3636        3645        3654        3663        3672
TAC ATG TGT TTA GTC GAG GTT AAA AAA CGT CTA GGC CCC CCG AAC CAC GGG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   M   C   L   V   E   V   K   K   R   L   G   P   P   N   H   G   D

     3681        3690        3699        3708        3717        3726
GTG GTT TTC CTT TGA AAA ACA CGA TAA TAC CAT GGG AAT TCA AGA TGG ATT GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   F   L   *   K   T   R   *   Y   H   G   N   S   R   W   I   A

     3735        3744        3753        3762        3771        3780
CGC AGG TTC TCC GGC CGC TTG GGT GGA GAG GCT ATT CGG CTA TGA CTG GGC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   R   F   S   G   R   L   G   G   E   A   I   R   L   *   L   G   T

     3789        3798        3807        3816        3825        3834
ACA GAC AAT CGG CTG CTC TGA TGC CGC CGT GTT CCG GCT GTC AGC GCA GGG GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   D   N   R   L   L   *   C   R   R   V   P   A   V   S   A   G   A

     3843        3852        3861        3870        3879        3888
CCC GGT TCT TTT TGT CAA GAC CGA CCT GTC CGG TGC CCT GAA TGA ACT GCA GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   G   S   F   C   Q   D   R   P   V   R   C   P   E   *   T   A   G

     3897        3906        3915        3924        3933        3942
CGA GGC AGC GCG GCT ATC GTG GCT GGC CAC GAC GGG CGT TCC TTG CGC AGC TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   G   S   A   A   I   V   A   G   H   D   G   R   S   L   R   S   C

     3951        3960        3969        3978        3987        3996
GCT CGA CGT TGT CAC TGA AGC GGG AAG GGA CTG GCT GCT ATT GGG CGA AGT GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

FIGURE 20-8


```
A   R   R   C   H   *       G   K   G   L   A   A   I       G   R   S   A

      4005        4014        4023        4032        4041        4050
GGG GCA GGA TCT CCT GTC ATC TCA CCT TGC TCC TGC CGA GAA AGT ATC CAT CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   A   G   S   P   V   I   S   P   C   S   C   R   E   S   I   H   H

      4059        4068        4077        4086        4095        4104
GGC TGA TGC AAT GCG GCG GCT GCA TAC GCT TGA TCC GGC TAC CTG CCC ATT CGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   *   C   N   A   A   A   A   Y   A   *   S   G   Y   L   P   I   R

      4113        4122        4131        4140        4149        4158
CCA CCA AGC GAA ACA TCG CAT CGA GCG AGC ACG TAC TCG GAT GGA AGC CGG TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   P   S   E   T   S   H   R   A   S   T   Y   S   D   G   S   R   S

      4167        4176        4185        4194        4203        4212
TGT CGA TCA GGA TGA TCT GGA CGA AGA GCA TCA GGG GCT CGC GCC AGC CGA ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   R   S   G   *   S   G   R   R   A   S   G   A   R   A   S   R   T

      4221        4230        4239        4248        4257        4266
GTT CGC CAG GCT CAA GGC GCG CAT GCC CGA CGG CGA GGA TCT CGT CGT GAC CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   R   Q   A   Q   G   A   H   A   R   R   R   G   S   R   R   D   P

      4275        4284        4293        4302        4311        4320
TGG CGA TGC CTG CTT GCC GAA TAT CAT GGT GGA AAA TGG CCG CTT TTC TGG ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   R   C   L   L   A   E   Y   H   G   G   K   W   P   L   F   W   I

      4329        4338        4347        4356        4365        4374
CAT CGA CTG TGG CCG GCT GGG TGT GGC GGA CCG CTA TCA GGA CAT AGC GTT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   R   L   W   P   A   G   C   G   G   P   L   S   G   H   S   V   G

      4383        4392        4401        4410        4419        4428
TAC CCG TGA TAT TGC TGA AGA GCT TGG CGG CGA ATG GGC TGA CCG CTT CCT CGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   P   *   Y   C   *   R   A   W   R   R   M   G   *   P   L   P   R

      4437        4446        4455        4464        4473        4482
GCT TTA CGG TAT CGC CGC TCC CGA TTC GCA GCG CAT CGC CTT CTA TCG CCT TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   R   Y   R   R   S   R   F   A   A   H   R   L   L   S   P   S

      4491        4500        4509        4518        4527        4536
TGA CGA GTT CTT CTG AGC GGG ACT CTG GGG ATC CGA TAA AAT AAA AGA TTT TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 *   R   V   L   L   S   G   T   L   G   I   R   *   N   K   R   F   Y

      4545        4554        4563        4572        4581        4590
```

FIGURE 20-9 **28/44**

```
TTA GTC TCC AGA AAA AGG GGG GAA TGA AAG ACC CCA CCT G[illegible] GGT TTG GCA AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   V   S   R   K   R   G   E   *   K   T   P   P   V   G   L   A   S

        4599        4608        4617        4626        4635        4644
TAG CTT AAG TAA CGC CAT TTT GCA AGG CAT GGA AAA ATA CAT AAC TGA GAA TAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 *   L   K   *   R   H   F   A   R   H   G   K   I   H   N   *   E   *

        4653        4662        4671        4680        4689        4698
AGA AGT TCA GAT CAA GGT CAG GAA CAG ATG GAA CAG CTG AAT ATG GGC CAA ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   S   S   D   Q   G   Q   E   Q   M   E   Q   L   N   M   G   Q   T

        4707        4716        4725        4734        4743        4752
GGA TAT CTG TGG TAA GCA GTT CCT GCC CCG GCT CAG GGC CAA GAA CAG ATG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   Y   L   W   *   A   V   P   A   P   A   Q   G   Q   E   Q   M   E

        4761        4770        4779        4788        4797        4806
CAG CTG AAT ATG GGC CAA ACA GGA TAT CTG TGG TAA GCA GTT CCT GCC CCG GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   L   N   M   G   Q   T   G   Y   L   W   *   A   V   P   A   P   A

        4815        4824        4833        4842        4851        4860
CAG GGC CAA GAA CAG ATG GTC CCC AGA TGC GGT CCA GCC CTC AGC AGT TTC TAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   G   Q   E   Q   M   V   P   R   C   G   P   A   L   S   S   F   *

        4869        4878        4887        4896        4905        4914
AGA ACC ATC AGA TGT TTC CAG GGT GCC CCA AGG ACC TGA AAT GAC CCT GTG CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   T   I   R   C   F   Q   G   A   P   R   T   *   N   D   P   V   P

        4923        4932        4941        4950        4959        4968
TAT TTG AAC TAA CCA ATC AGT TCG CTT CTC GCT TCT GTT CGC GCG CTT CTG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   L   N   *   P   I   S   S   L   L   A   S   V   R   A   L   L   L

        4977        4986        4995        5004        5013        5022
CCC GAG CTC AAT AAA AGA GCC CAC AAC CCC TCA CTC GGG GCG CCA GTC CTC CGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   E   L   N   K   R   A   H   N   P   S   L   G   A   P   V   L   R

        5031        5040        5049        5058        5067        5076
TTG ACT GAG TCG CCC GGG TAC CCG TGT ATC CAA TAA ACC CTC TTG CAG TTG CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   E   S   P   G   Y   P   C   I   Q   *   T   L   L   Q   L   H

        5085        5094        5103        5112        5121        5130
CCG ACT TGT GGT CTC GCT GTT CCT TGG GAG GGT CTC CTC TGA GTG ATT GAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   T   C   G   L   A   V   P   W   E   G   L   L   *   V   I   D   Y
```

FIGURE 20-10 29/44

```
        5139            5148            5157            5166            5175            5184
CCG TCA GCG GGG GTC TTT CAT TTG GGG GCT CGT CCG GGA TCG GGA GAC CCC TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   A   G   V   F   H   L   G   A   R   P   G   S   G   D   P   C

        5193            5202            5211            5220            5229            5238
CCA GGG ACC ACC GAC CCA CCA CCG GGA GGT AAG CTG GCT GCC TCG CGC GTT TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   G   T   T   D   P   P   P   G   G   K   L   A   A   S   R   V   S

        5247            5256            5265            5274            5283            5292
GTG ATG ACG GTG AAA ACC TCT GAC ACA TGC AGC TCC CGG AGA CGG TCA CAG CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   M   T   V   K   T   S   D   T   C   S   S   R   R   R   S   Q   L

        5301            5310            5319            5328            5337            5346
GTC TGT AAG CGG ATG CCG GGA GCA GAC AAG CCC GTC AGG GCG CGT CAG CGG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   C   K   R   M   P   G   A   D   K   P   V   R   A   R   Q   R   V

        5355            5364            5373            5382            5391            5400
TTG GCG GGT GTC GGG GCG CAG CCA TGA CCC AGT CAC GTA GCG ATA GCG GAG TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   A   G   V   G   A   Q   P   *   P   S   H   V   A   I   A   E   C

        5409            5418            5427            5436            5445            5454
ATA CTG GCT TAA CTA TGC GGC ATC AGA GCA GAT TGT ACT GAG AGT GCA CCA TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   L   A   *   L   C   G   I   R   A   D   C   T   E   S   A   P   Y

        5463            5472            5481            5490            5499            5508
GCG GTG TGA AAT ACC GCA CAG ATG CGT AAG GAG AAA ATA CCG CAT CAG GCG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   V   *   N   T   A   Q   M   R   K   E   K   I   P   H   Q   A   L

        5517            5526            5535            5544            5553            5562
TTC CGC TTC CTC GCT CAC TGA CTC GCT GCG CTC GGT CGT TCG GCT GCG GCG AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   R   F   L   A   H   *   L   A   A   L   G   R   S   A   A   A   S

        5571            5580            5589            5598            5607            5616
GGT ATC AGC TCA CTC AAA GGC GGT AAT ACG GTT ATC CAC AGA ATC AGG GGA TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   I   S   S   L   K   G   G   N   T   V   I   H   R   I   R   G   *

        5625            5634            5643            5652            5661            5670
CGC AGG AAA GAA CAT GTG AGC AAA AGG CCA GCA AAA GGC CAG GAA CCG TAA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   R   K   E   H   V   S   K   R   P   A   K   G   Q   E   P   *   K

        5679            5688            5697            5706            5715            5724
GGC CGC GTT GCT GGC GTT TTT CCA TAG GCT CCG CCC CCC TGA CGA GCA TCA CAA
```

FIGURE 20-11 30/44

```
-- --- --                                        -- --- --- --- --
G   R   V   A   G   V   F   P   *   A   P   P   P       R   A   S   Q

        5733        5742        5751        5760        5769        5778
AAA TCG ACG CTC AAG TCA GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   S   T   L   K   S   E   V   A   K   P   D   R   T   I   K   I   P

        5787        5796        5805        5814        5823        5832
GGC GTT TCC CCC TGG AAG CTC CCT CGT GCG CTC TCC TGT TCC GAC CCT GCC GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   V   S   P   W   K   L   P   R   A   L   S   C   S   D   P   A   A

        5841        5850        5859        5868        5877        5886
TAC CGG ATA CCT GTC CGC CTT TCT CCC TTC GGG AAG CGT GGC GCT TTC TCA ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   R   I   P   V   R   L   S   P   F   G   K   R   G   A   F   S   M

        5895        5904        5913        5922        5931        5940
CTC ACG CTG TAG GTA TCT CAG TTC GGT GTA GGT CGT TCG CTC CAA GCT GGG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   L   *   V   S   Q   F   G   V   G   R   S   L   Q   A   G   L

        5949        5958        5967        5976        5985        5994
TGT GCA CGA ACC CCC CGT TCA GCC CGA CCG CTG CGC CTT ATC CGG TAA CTA TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   A   R   T   P   R   S   A   R   P   L   R   L   I   R   *   L   S

        6003        6012        6021        6030        6039        6048
TCT TGA GTC CAA CCC GGT AAG ACA CGA CTT ATC GCC ACT GGC AGC AGC CAC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   *   V   Q   P   G   K   T   R   L   I   A   T   G   S   S   H   W

        6057        6066        6075        6084        6093        6102
TAA CAG GAT TAG CAG AGC GAG GTA TGT AGG CGG TGC TAC AGA GTT CTT GAA GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 *   Q   D   *   Q   S   E   V   C   R   R   C   Y   R   V   L   E   V

        6111        6120        6129        6138        6147        6156
GTG GCC TAA CTA CGG CTA CAC TAG AAG GAC AGT ATT TGG TAT CTG CGC TCT GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   A   *   L   R   L   H   *   K   D   S   I   W   Y   L   R   S   A

        6165        6174        6183        6192        6201        6210
GAA GCC AGT TAC CTT CGG AAA AAG AGT TGG TAG CTC TTG ATC CGG CAA ACA AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   A   S   Y   L   R   K   K   S   W   *   L   L   I   R   Q   T   N

        6219        6228        6237        6246        6255        6264
CAC CGC TGG TAG CGG TGG TTT TTT TGT TTG CAA GCA GCA GAT TAC GCG CAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   R   W   *   R   W   F   F   C   L   Q   A   A   D   Y   A   Q   K
```

FIGURE 20-12

```
        6273           6282           6291           6300           6309           6318
AAA AGG ATC TCA AGA AGA TCC TTT GAT CTT TTC TAC GGG GT[illegible] TGA CGC TCA GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   R   I   S   R   R   S   F   D   L   F   Y   G   V   *   R   S   V

        6327           6336           6345           6354           6363           6372
GAA CGA AAA CTC ACG TTA AGG GAT TTT GGT CAT GAG ATT ATC AAA AAG GAT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   R   K   L   T   L   R   D   F   G   H   E   I   I   K   K   D   L

        6381           6390           6399           6408           6417           6426
CAC CTA GAT CCT TTT AAA TTA AAA ATG AAG TTT TAA ATC AAT CTA AAG TAT ATA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   L   D   P   F   K   L   K   M   K   F   *   I   N   L   K   Y   I

        6435           6444           6453           6462           6471           6480
TGA GTA AAC TTG GTC TGA CAG TTA CCA ATG CTT AAT CAG TGA GGC ACC TAT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 *   V   N   L   V   *   Q   L   P   M   L   N   Q   *   G   T   Y   L

        6489           6498           6507           6516           6525           6534
AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT TGC CTG ACT CCC CGT CGT GTA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   D   L   S   I   S   F   I   H   S   C   L   T   P   R   R   V   D

        6543           6552           6561           6570           6579           6588
AAC TAC GAT ACG GGA GGG CTT ACC ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   Y   D   T   G   G   L   T   I   W   P   Q   C   C   N   D   T   A

        6597           6606           6615           6624           6633           6642
AGA CCC ACG CTC ACC GGC TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CGG AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   P   T   L   T   G   S   R   F   I   S   N   K   P   A   S   R   K

        6651           6660           6669           6678           6687           6696
GGC CGA GCG CAG AAG TGG TCC TGC AAC TTT ATC CGC CTC CAT CCA GTC TAT TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   R   A   Q   K   W   S   C   N   F   I   R   L   H   P   V   Y   *

        6705           6714           6723           6732           6741           6750
TTG TTG CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   L   P   G   S   *   S   K   *   F   A   S   *   *   F   A   Q   R

        6759           6768           6777           6786           6795           6804
TGT TGC CAT TGC TGC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT GGC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   C   H   C   C   R   H   R   G   V   T   L   V   V   W   Y   G   F

        6813           6822           6831           6840           6849           6858
ATT CAG CTC CGG TTC CCA ACG ATC AAG GCG AGT TAC ATG ATC CCC CAT GTT GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

FIGURE 20-13 *32/44*

```
 I   Q   L   R   F   P   T   I   K   A   S   Y   M  [illegible] P   H   V   V

        6867        6876       6885        6894        6903        6912
CAA AAA AGC GGT TAG CTC CTT CGG TCC TCC GAT CGT TGT CAG AAG TAA GTT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   K   S   G   *   L   L   R   S   S   D   R   C   Q   K   *   V   G

        6921        6930        6939        6948        6957        6966
CGC AGT GTT ATC ACT CAT GGT TAT GGC AGC ACT GCA TAA TTC TCT TAC TGT CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   S   V   I   T   H   G   Y   G   S   T   A   *   F   S   Y   C   H

        6975        6984        6993        7002        7011        7020
GCC ATC CGT AAG ATG CTT TTC TGT GAC TGG TGA GTA CTC AAC CAA GTC ATT CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   I   R   K   M   L   F   C   D   W   *   V   L   N   Q   V   I   L

        7029        7038        7047        7056        7065        7074
AGA ATA GTG TAT GCG GCG ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   I   V   Y   A   A   T   E   L   L   L   P   G   V   N   T   G   *

        7083        7092        7101        7110        7119        7128
TAC CGC GCC ACA TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   R   A   T   *   Q   N   F   K   S   A   H   H   W   K   T   F   F

        7137        7146        7155        7164        7173        7182
GGG GCG AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   A   K   T   L   K   D   L   T   A   V   E   I   Q   F   D   V   T

        7191        7200        7209        7218        7227        7236
CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT TAC TTT CAC CAG CGT TTC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   S   C   T   Q   L   I   F   S   I   F   Y   F   H   Q   R   F   W

        7245        7254        7263        7272        7281        7290
GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GGG AAT AAG GGC GAC ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   S   K   N   R   K   A   K   C   R   K   K   G   N   K   G   D   T

        7299        7308        7317        7326        7335        7344
GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA TTA TTG AAG CAT TTA TCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   M   L   N   T   H   T   L   P   F   S   I   L   L   K   H   L   S

        7353        7362        7371        7380        7389        7398
GGG TTA TTG TCT CAT GAG CGG ATA CAT ATT TGA ATG TAT TTA GAA AAA TAA ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   L   S   H   E   R   I   H   I   *   M   Y   L   E   K   *   T

        7407        7416        7425        7434        7443        7452
```

FIGURE 20-14


```
AAT AGG GGT TCC GCG CAC [illegible]T TCC CCG AAA AGT GCC ACC T[illegible] CGT CTA AGA AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   R   G   S   A   H   I   S   P   K   S   A   T   *   R   L   R   N

     7461        7470        7479        7488        7497        7506
CAT TAT TAT CAT GAC ATT AAC CTA TAA AAA TAG GCG TAT CAC GAG GCC CTT TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   Y   Y   H   D   I   N   L   *   K   *   A   Y   H   E   A   L   S

     7515        7524        7533        7542        7551        7560
TCT TCA AGA ATT CAT ACC AGA TCA CCG AAA ACT GTC CTC CAA ATG TGT CCC CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   S   R   I   H   T   R   S   P   K   T   V   L   Q   M   C   P   P

     7569        7578        7587        7596        7605        7614
CAC ACT CCC AAA TTC GCG GGC TTC TGC TCT TAG ACC ACT CTA CCC TAT TCC CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   T   P   K   F   A   G   F   C   S   *   T   T   L   P   Y   S   P

     7623        7632        7641        7650        7659        7668
CAC TCA CCG GAG CCA AAG CCG CGG CCC TTC CGT TTC TTT GCT TTT GAA AGA CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   S   P   E   P   K   P   R   P   F   R   F   F   A   F   E   R   P

     7677        7686        7695        7704        7713        7722
CAC CCG TAG GTG GCA AGC TAG CTT AAG TAA CGC CAC TTT GCA AGG CAT GGA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   P   *   V   A   S   *   L   K   *   R   H   F   A   R   H   G   K

     7731        7740        7749        7758        7767        7776
ATA CAT AAC TGA GAA TAG GAA AGT TCA GAT CAA GGT CAG GAA CAA AGA AAC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   H   N   *   E   *   E   S   S   D   Q   G   Q   E   Q   R   N   S

     7785        7794        7803        7812        7821        7830
TGA ATA CCA AAC AGG ATA TCT GTG GTA AGC GGT TCC TGC CCC GGC TCA GGG CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 *   I   P   N   R   I   S   V   V   S   G   S   C   P   G   S   G   P

     7839        7848        7857        7866        7875        7884
AGA ACA GAT GAG ACA GCT GAG TGA TGG GCC AAA CAG GAT ATC TGT GGT AAG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   T   D   E   T   A   E   *   W   A   K   Q   D   I   C   G   K   Q

     7893        7902        7911        7920        7929        7938
TTC CTG CCC CGG CTC GGG GCC AAG AAC AGA TGG TCC CCA GAT GCG GTC CAG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   L   P   R   L   G   A   K   N   R   W   S   P   D   A   V   Q   P

     7947        7956        7965        7974        7983        7992
TCA GCA GTT TCT AGT GAA TCA TCA GAT GTT TCC AGG GTG CCC CAA GGA CCT GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   A   V   S   S   E   S   S   D   V   S   R   V   P   Q   G   P   E
```

FIGURE 20-15 *34/44*

```
        8001              8010              8019              8028              8037              8046
AAT GAC CCT GTA CCT TAT TTG AAC TAA CCA ATC AGT TCG CTT CTC GCT TCT GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   D   P   V   P   Y   L   N   *   P   I   S   S   L   L   A   S   V

        8055              8064              8073
CGC GCG CTT CCG CTC TCC GAG CTC AAT AAA AG 3'
--- --- --- --- --- --- --- --- --- --- --
 R   A   L   P   L   S   E   L   N   K
```

FIGURE 21

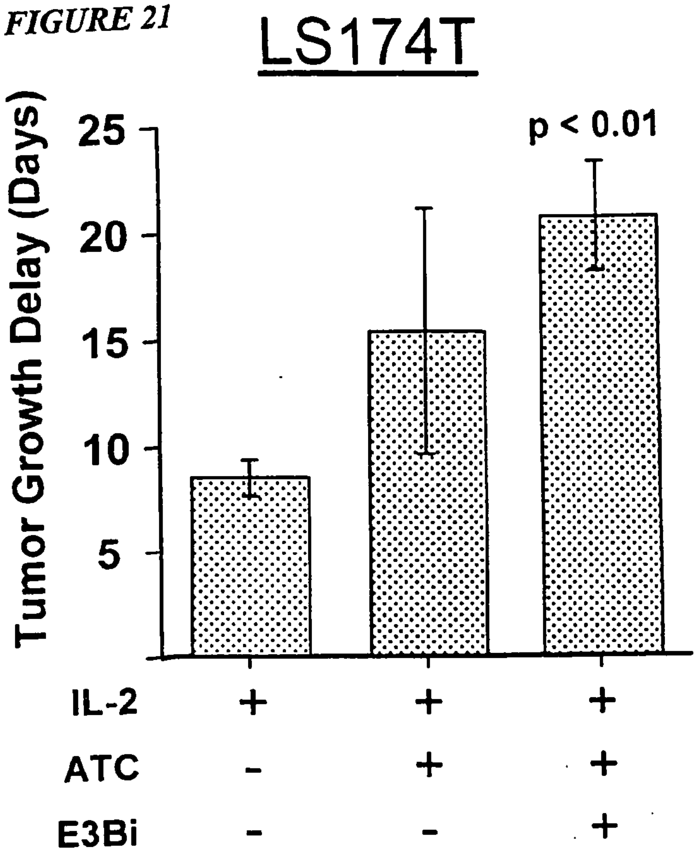


Figure 9. E3Bi induces ATC to produce significant tumor growth delay in mice. SCID-Beige mice bearing LS174T xenografts were treated i.t. with IL-2 (n=6), or IL-2/ATC (n=8), or IL-2/ATC/E3Bi (n=6) beginning when tumor volumes of mice reached approximately 0.5 cc. Tumor growth delay is reported as the mean number of days (±SD) for tumor volumes of mice from each treatment group to reach 2 cc. P =0.0034 is the probability by Kruskal-Wallis non-parametric analysis that tumor growth delay is the same for all treatment groups. $P < 0.01$ is the probability by Dunn's multiple comparison analysis that treatment with IL-2/ATC/E3BI produces the same tumor growth delay in mice as treatment with IL-2 alone; P>0.05 for IL-2/ATC alone.

FIGURE 22

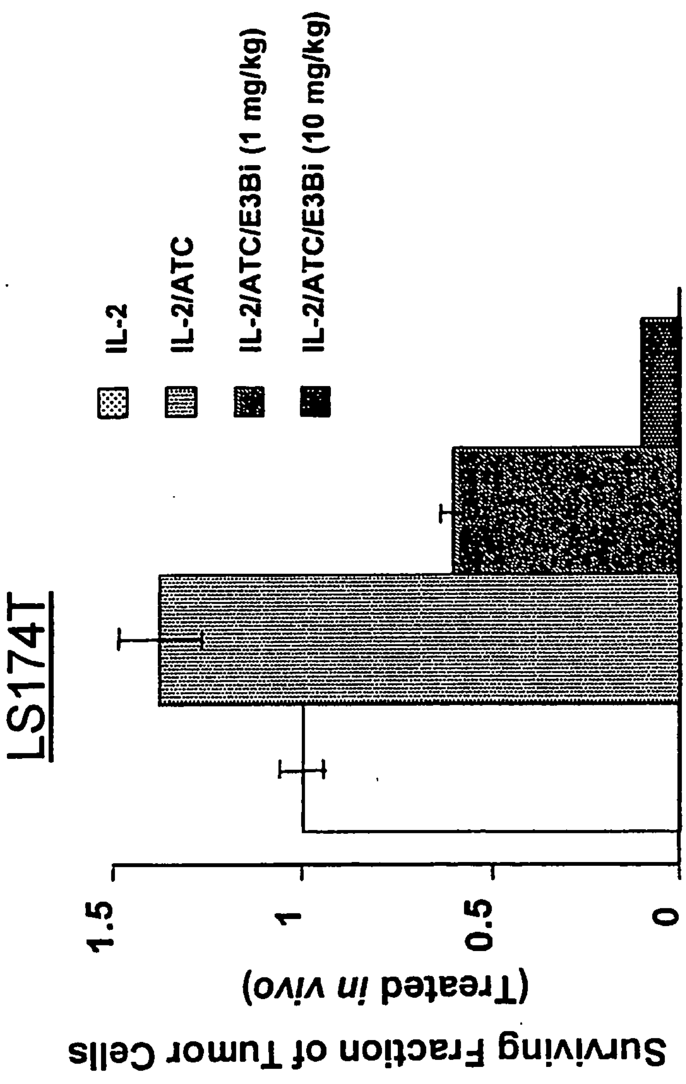

LS174T
IL-2
IL-2/ATC
IL-2/ATC/E3Bi (1 mg/kg)
IL-2/ATC/E3Bi (10 mg/kg)
Surviving Fraction of Tumor Cells
(Treated in vivo)
1.5
1
0.5
0
1
2
3
4

FIGURE 23

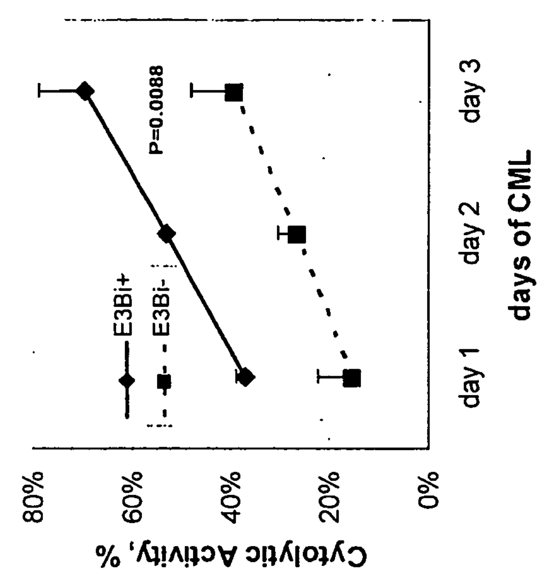

Cytolytic Activity, %
80%
60%
40%
20%
0%
E3Bi+
E3Bi-
P=0.0088
day 1
day 2
day 3
days of CML

FIGURE 24 E3Bi cDNA Sequence

ATGGATTTTC AGGTGCAGAT TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCT
AGAGGGAGCA TTGTAATGAC CCAATCTCAC AAATTCATGT CCACATCAGT AGGAGACAGT
GTCAGCATCA CCTGCAAGGC CAGTCAGGAT GTGAGTACTG CTGTAGCCTG GTATCAACAG
AAACCAGGAC AATCTCCTAA ACTACTGATT TACTCGGCAT CCGACCGGTA CACTGGAGTC
CCTGATCGCT TCACTGGCAG TGGATCTGGG ACGGATTTCA CTTTCACCAT CAGCAGTGTG
CAGGCTGAAG ACCTGGCAGT TTATTACTGT CACCAACATT ATATTACTCC TCGGACGTTC
GGTGGAGGCA CAAAGCTGGA AATAAAAGGG TCGACTTCCG GTAGCGGCAA ATCCTCTGAA
GGCAAAGGTC AGGTCCAGCT GCAGCAGTCT GGAGCTGAGG TGATGAGGCC TGGGGCCTCA
GTGAAGATAT CCTGCAAGGC TACTGGCTAC ACATTCACTA GGTACTACAT ACAATGGGGT
AAAAACAGGC CTGGACATGG CCTTGAGTGG ATTGGAGAGA TTTTACCTGG AACTCTTACT
AATTACAATG AGAAATTCAA GGGCAAGGCC GCATTCACTG CAGATAGATC CTCCAACACA
GCCTACATGC AACTCAGCAG CCTTACATCT GAGGACTCTG CCGTCTATTA CTGTGCAAGA
GATGGTCCCT GGTTTGCTTA CTGGGGCCAA GGAACCCTGG TCACCGTCTC TGCAGCGGAT
CTGAGCAACT CCATCATGTA CTTCAGCCAC TTCGTGCCGG TCTTCCTGCC AGCGAAGCCC
ACCACGACGC CAGCGCCGCG ACCACCAACA CCGGCGCCCA CCATCGCGTC GCAGCCCCTG
TCCCTGCGCC CAGAGGCGTG CCGGCCAGCG GCGGGGGGCG CAGTCCACAC GAGGGGGCTG
GACTTCGCGG ATCCACAGGT CCAGCTACAG CAGTCTGGGG CTGAACTGGC AAGACCTGGG
GCCTCAGTGA AGATGTCCTG CAAGGCTTCT GGCTACACCT TTACTAGGTA CACGATGCAC
TGGGTAAAAC AGAGGCCTGG ACAGGGTCTG GAATGGATTG GATACATTAA TCCTAGCCGT
GGTTATACTA ATTACAATCA GAAGTTCAAG GACAAGGCCA CATTGACTAC AGACAAATCC
TCCAGCACAG CCTACATGCA ACTGAGCAGC CTGACATCTG AGGACTCTGC AGTCTATTAC
TGTGCAAGAT ATTATGATGA TCATTACTGC CTTGACTACT GGGGCCAAGG CACCACTCTC
ACAGTCTCCT CAGGATCTAC TTCAGGTAGC GGTAAATCAT CTGAAGGTAA AGGTCAGGTC
CTCCAAATTG TTCTCACCCA GTCTCCAGCA ATCATGTCTG CATCTCCAGG GGAGAAGGTC
ACCATGACCT GCAGTGCCAG CTCAAGTGTA AGTTACATGA ACTGGTACCA GCAGAAGTCA
GGCACCTCCC CCAAAAGATG GATTTATGAC ACATCCAAAC TGGCTTCTGG AGTCCCTGCT
CACTTCAGGG GCAGTGGGTC TGGGACCTCT TACTCTCTCA CAATCAGCGG CATGGAGGCT
GAAGATGCTG CCACTTATTA CTGCCAGCAG TGGAGTAGTA ACCCATTCAC GTTCGGCTCG
GGGACAAAGT TGGAAATAAA CCGGCACCAT CACCATCACC ATTAGACTCG A

FIGURE 25

Protein sequence of E3Bi

MDFQVQIFSFLLISASVIMSRGSIVMTQSHKFMSTSVGDSVSITCKASQDVSTAVAWYQQ
KPGQSPKLLIYSASDRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCHQHYITPRTF
GGGTKLEIKGSTSGSGKSSEGKGQVQLQQSGAEVMRPGASVKISCKATGYTFTRYYIQWG
KNRPGHGLEWIGEILPGTLTNYNEKFKGKAAFTADRSSNTAYMQLSSLTSEDSAVYYCAR
DGPWFAYWGQGTLVTVSAADLSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPL
SLRPEACRPAAGGAVHTRGLDFADPQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMH
WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYY
CARYYDDHYCLDYWGQGTTLTVSSGSTSGSGKSSEGKGQVLQIVLTQSPAIMSASPGEKV
TMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEA
EDAATYYCQQWSSNPFTFGSGTKLEINRHHHHHH*

FIGURE 26-1 **The Sequence of pE3Bi**

(8078 residue sequence starting "AGCCCACAAC")

```
   1 S  P  Q  P  L  T  R  R  A  S  L  P  I  D  C  V  A  R  V  P
   1 AGCCCACAACCCCTCACTCGGCGCGCCAGTCTTCCGATAGACTGCGTCGCCCGGGTACCC
  21 V  F  P  I  K  P  L  A  V  C  I  R  I  V  V  S  L  F  L  G
  61 GTATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGTCTCGCTGTTCCTTGGG
  41 R  V  S  S  E  *  L  T  T  H  D  G  G  L  S  F  G  G  S  S
 121 AGGGTCTCCTCTGAGTGATTGACTACCCACGACGGGGGTCTTTCATTTGGGGGCTCGTCC
  61 G  I  W  R  P  L  P  R  D  H  R  P  T  T  G  R  *  A  G  Q
 181 GGGATTTGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCCAG
  81 Q  P  I  C  V  C  P  I  V  *  C  L  C  L  M  L  C  A  C  V
 241 CAACCTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGTTTGATGTTATGCGCCTGCGTC
 101 C  T  S  *  L  T  S  S  V  S  G  G  P  V  V  E  L  T  S  S
 301 TGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCT
 121 E  H  P  A  A  T  Q  G  D  V  P  G  T  L  G  A  V  F  V  A
 361 GAACACCCGGCCGCAACCCAGGGAGACGTCCCAGGGACTTTGGGGGCCGTTTTTGTGGCC
 141 R  P  E  E  G  S  R  C  G  I  R  P  R  Q  D  M  W  F  W  *
 421 CGACCTGAGGAAGGGAGTCGATGTGGAATCCGACCCCGTCAGGATATGTGGTTCTGGTAG
 161 E  T  R  T  *  N  S  S  R  L  R  L  N  F  C  F  R  F  G  T
 481 GAGACGAGAACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGAACC
 181 E  A  A  R  L  V  C  C  S  I  V  L  C  C  L  C  L  T  V  F
 541 GAAGCCGCGCGTCTTGTCTGCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTT
 201 L  Y  L  S  E  N  *  G  Q  T  V  T  T  P  L  S  L  T  L  G
 601 CTGTATTTGTCTGAAAATTAGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGT
 221 H  W  K  D  V  E  R  I  A  H  N  Q  S  V  D  V  K  K  R  R
 661 CACTGGAAAGATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGT
 241 W  V  T  F  C  S  A  E  W  P  T  F  N  V  G  W  P  R  D  G
 721 TGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGC
 261 T  F  N  R  D  L  I  T  Q  V  K  I  K  V  F  S  P  G  P  H
 781 ACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCAT
 281 G  H  P  D  Q  V  P  Y  I  V  T  W  E  A  L  A  F  D  P  P
 841 GGACACCCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCT
 301 P  W  V  K  P  F  V  H  P  K  P  P  P  P  L  P  P  S  A  P
 901 CCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCG
 321 S  L  P  L  E  P  P  R  S  T  P  P  R  S  S  L  Y  P  A  L
 961 TCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTC
 341 T  P  S  L  G  A  G  I  R  G  R  D  K  S  Y  *  Q  P  L  S
1021 ACTCCTTCTCTAGGCGCCGGAATTCGCGGCCGTGACAAGAGTTACTAACAGCCCCTCTCT
 361 P  S  S  L  T  G  S  L  L  S  P  A  R  S  L  E  T  S  G  G
1081 CCAAGCTCACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGC
 381 S  L  P  R  T  T  G  P  T  G  G  T  S  P  L  P  S  R  R  H
1141 AGCCTACCAAGAACAACTGGACCGACCGGTGGTACCTCACCCTTACCGAGTCGGCGACAC
 401 S  V  G  P  P  T  P  D  *  E  P  R  T  S  L  E  R  T  L  H
1201 AGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGGAAAGGACCTTACAC
 421 S  P  A  D  H  P  H  R  P  Q  S  R  R  H  R  S  L  D  T  R
1261 AGTCCTGCAGACCACCCCCACCGCCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGC
 441 R  P  R  E  G  C  R  P  R  G  W  T  I  S  R  L  T  R  P  L
1321 CGCCCACGTGAAGGCTGCCGACCCCGGGGGTGGACCATCTCTAGACTGACGCGGCCGCTA
 461 R  T  M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S  V  I
1381 CGTACCATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATA
 481 M  S  R  G  S  I  V  M  T  Q  S  H  K  F  M  S  T  S  V  G
1441 ATGTCTAGAGGGAGCATTGTAATGACCCAATCTCACAAATTCATGTCCACATCAGTAGGA
 501 D  S  V  S  I  T  C  K  A  S  Q  D  V  S  T  A  V  A  W  Y
1501 GACAGTGTCAGCATCACCTGCAAGGCCAGTCAGGATGTGAGTACTGCTGTAGCCTGGTAT
 521 Q  Q  K  P  G  Q  S  P  K  L  L  I  Y  S  A  S  D  R  Y  T
```

FIGURE 26-2 *41/44*

```
1561 CAACAGAAACCAGGACAATCTCCTAAACTACTGATTTACTCGGCATCCGACCGGTACACT
 541 G  V  P  D  R  F  T  G  S  G  S  G  T  D  F  T  F  T  I  S
1621 GGAGTCCCTGATCGCTTCACTGGCAGTGGATCTGGGACGGATTTCACTTTCACCATCAGC
 561 S  V  Q  A  E  D  L  A  V  Y  Y  C  H  Q  H  Y  I  T  P  R
1681 AGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCACCAACATTATATTACTCCTCGG
 581 T  F  G  G  G  T  K  L  E  I  K  G  S  T  S  G  S  G  K  S
1741 ACGTTCGGTGGAGGCACAAAGCTGGAAATAAAAGGGTCGACTTCCGGTAGCGGCAAATCC
 601 S  E  G  K  G  Q  V  Q  L  Q  Q  S  G  A  E  V  M  R  P  G
1801 TCTGAAGGCAAAGGTCAGGTCCAGCTGCAGCAGTCTGGAGCTGAGGTGATGAGGCCTGGG
 621 A  S  V  K  I  S  C  K  A  T  G  Y  T  F  T  R  Y  Y  I  Q
1861 GCCTCAGTGAAGATATCCTGCAAGGCTACTGGCTACACATTCACTAGGTACTACATACAA
 641 W  G  K  N  R  P  G  H  G  L  E  W  I  G  E  I  L  P  G  T
1921 TGGGGTAAAAACAGGCCTGGACATGGCCTTGAGTGGATTGGAGAGATTTTACCTGGAACT
 661 L  T  N  Y  N  E  K  F  K  G  K  A  A  F  T  A  D  R  S  S
1981 CTTACTAATTACAATGAGAAATTCAAGGGCAAGGCCGCATTCACTGCAGATAGATCCTCC
 681 N  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A  V  Y  Y  C
2041 AACACAGCCTACATGCAACTCAGCAGCCTTACATCTGAGGACTCTGCCGTCTATTACTGT
 701 A  R  D  G  P  W  F  A  Y  W  G  Q  G  T  L  V  T  V  S  A
2101 GCAAGAGATGGTCCCTGGTTTGCTTACTGGGGCCAAGGAACCCTGGTCACCGTCTCTGCA
 721 A  D  L  S  N  S  I  M  Y  F  S  H  F  V  P  V  F  L  P  A
2161 GCGGATCTGAGCAACTCCATCATGTACTTCAGCCACTTCGTGCCGGTCTTCCTGCCAGCG
 741 K  P  T  T  T  P  A  P  R  P  P  T  P  A  P  T  I  A  S  Q
2221 AAGCCCACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAG
 761 P  L  S  L  R  P  E  A  C  R  P  A  A  G  G  A  V  H  T  R
2281 CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTCCACACGAGG
 781 G  L  D  F  A  D  P  Q  V  Q  L  Q  Q  S  G  A  E  L  A  R
2341 GGGCTGGACTTCGCGGATCCACAGGTCCAGCTACAGCAGTCTGGGGCTGAACTGGCAAGA
 801 P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T  R  Y  T
2401 CCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACCTTTACTAGGTACACG
 821 M  H  W  V  K  Q  R  P  G  Q  G  L  E  W  I  G  Y  I  N  P
2461 ATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGATTGGATACATTAATCCT
 841 S  R  G  Y  T  N  Y  N  Q  K  F  K  D  K  A  T  L  T  T  D
2521 AGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGAC
 861 K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A  V
2581 AAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTC
 881 Y  Y  C  A  R  Y  Y  D  D  H  Y  C  L  D  Y  W  G  Q  G  T
2641 TATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGCCAAGGCACC
 901 T  L  T  V  S  S  G  S  T  S  G  S  G  K  S  S  E  G  K  G
2701 ACTCTCACAGTCTCCTCAGGATCTACTTCAGGTAGCGGTAAATCATCTGAAGGTAAAGGT
 921 Q  V  Q  Q  I  V  L  T  Q  S  P  A  I  M  S  A  S  P  G  E
2761 CAGGTCCAGCAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAG
 941 K  V  T  M  T  C  S  A  S  S  S  V  S  Y  M  N  W  Y  Q  Q
2821 AAGGTCACCATGACCTGCAGTGCCAGCTCAAGTGTAAGTTACATGAACTGGTACCAGCAG
 961 K  S  G  T  S  P  K  R  W  I  Y  D  T  S  K  L  A  S  G  V
2881 AAGTCAGGCACCTCCCCCAAAAGATGGATTTATGACACATCCAAACTGGCTTCTGGAGTC
 981 P  A  H  F  R  G  S  G  S  G  T  S  Y  S  L  T  I  S  G  M
2941 CCTGCTCACTTCAGGGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCGGCATG
1001 E  A  E  D  A  A  T  Y  Y  C  Q  Q  W  S  S  N  P  F  T  F
3001 GAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGAGTAGTAACCCATTCACGTTC
1021 G  S  G  T  K  L  E  I  N  R  H  H  H  H  H  H  *  T  R  G
3061 GGCTCGGGGACAAAGTTGGAAATAAACCGGCACCATCACCATCACCATTAGACTCGAGGA
1041 S  I  P  P  L  S  L  P  P  P  *  R  Y  W  P  K  P  L  G  I
3121 TCAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATA
1061 R  P  V  C  V  C  L  Y  V  I  F  H  H  I  A  V  F  W  Q  C
3181 AGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGT
1081 E  G  P  E  T  W  P  C  L  L  D  E  H  S  *  G  S  F  P  S
3241 GAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCT
```

FIGURE 26-3 *42/44*

```
1101 R  Q  R  N  A  R  S  V  E  C  R  E  G  S  S  S  S  G  S  F
3301 CGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTC
1121 L  K  T  N  N  V  C  S  D  P  L  Q  A  A  E  P  P  T  W  R
3361 TTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGA
1141 Q  V  P  L  R  P  K  A  T  C  I  R  Y  T  C  K  G  G  T  T
3421 CAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACC
1161 P  V  P  R  C  E  L  D  S  C  G  K  S  Q  M  A  L  L  K  R
3481 CCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGT
1181 I  Q  Q  G  A  E  G  C  P  E  G  T  P  L  Y  G  I  *  S  G
3541 ATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGG
1201 A  S  V  H  M  L  Y  M  C  L  V  E  V  K  K  R  L  G  P  P
3601 GCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCG
1221 N  H  G  D  V  V  F  L  *  K  T  R  *  Y  H  G  N  S  R  W
3661 AACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATACCATGGGAATTCAAGATGG
1241 I  A  R  R  F  S  G  R  L  G  G  E  A  I  R  L  *  L  G  T
3721 ATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACA
1261 T  D  N  R  L  L  *  C  R  R  V  P  A  V  S  A  G  A  P  G
3781 ACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGT
1281 S  F  C  Q  D  R  P  V  R  C  P  E  *  T  A  G  R  G  S  A
3841 TCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCG
1301 A  I  V  A  G  H  D  G  R  S  L  R  S  C  A  R  R  C  H  *
3901 GCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGA
1321 S  G  K  G  L  A  A  I  G  R  S  A  G  A  G  S  P  V  I  S
3961 AGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCA
1341 P  C  S  C  R  E  S  I  H  H  G  *  C  N  A  A  A  A  Y  A
4021 CCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCT
1361 *  S  G  Y  L  P  I  R  P  P  S  E  T  S  H  R  A  S  T  Y
4081 TGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAC
1381 S  D  G  S  R  S  C  R  S  G  *  S  G  R  R  A  S  G  A  R
4141 TCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGC
1401 A  S  R  T  V  R  Q  A  Q  G  A  H  A  R  R  R  G  S  R  R
4201 GCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGT
1421 D  P  W  R  C  L  L  A  E  Y  H  G  G  K  W  P  L  F  W  I
4261 GACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATT
1441 H  R  L  W  P  A  G  C  G  G  P  L  S  G  H  S  V  G  Y  P
4321 CATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCG
1461 *  Y  C  *  R  A  W  R  R  M  G  *  P  L  P  R  A  L  R  Y
4381 TGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTAT
1481 R  R  S  R  F  A  A  H  R  L  L  S  P  S  *  R  V  L  L  S
4441 CGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGC
1501 G  T  L  G  I  R  *  N  K  R  F  Y  L  V  S  R  K  R  G  E
4501 GGGACTCTGGGGATCCGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAA
1521 *  K  T  P  P  V  G  L  A  S  *  L  K  *  R  H  F  A  R  H
4561 TGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCAT
1541 G  K  I  H  N  *  E  *  R  S  S  D  Q  G  Q  E  Q  M  E  Q
4621 GGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAG
1561 L  N  M  G  Q  T  G  Y  L  W  *  A  V  P  A  P  A  Q  G  Q
4681 CTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAA
1581 E  Q  M  E  Q  L  N  M  G  Q  T  G  Y  L  W  *  A  V  P  A
4741 GAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCC
1601 P  A  Q  G  Q  E  Q  M  V  P  R  C  G  P  A  L  S  S  F  *
4801 CCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAG
1621 R  T  I  R  C  F  Q  G  A  P  R  T  *  N  D  P  V  P  Y  L
4861 AGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTG
1641 N  *  P  I  S  S  L  L  A  S  V  R  A  L  L  L  P  E  L  N
4921 AACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAAT
1661 K  R  A  H  N  P  S  L  G  A  P  V  L  R  L  T  E  S  P  G
```

FIGURE 26-4 *43/44*

```
4981 AAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGG
1681 Y  P  C  I  Q  *  T  L  L  Q  L  H  P  T  C  G  L  A  V  P
5041 TACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCT
1701 W  E  G  L  L  *  V  I  D  Y  P  S  A  G  V  F  H  L  G  A
5101 TGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCT
1721 R  P  G  S  G  D  P  C  P  G  T  T  D  P  P  P  G  G  K  L
5161 CGTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTG
1741 A  A  S  R  V  S  V  M  T  V  K  T  S  D  T  C  S  S  R  R
5221 GCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGA
1761 R  S  Q  L  V  C  K  R  M  P  G  A  D  K  P  V  R  A  R  Q
5281 CGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAG
1781 R  V  L  A  G  V  G  A  Q  P  *  P  S  H  V  A  I  A  E  C
5341 CGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGT
1801 I  L  A  *  L  C  G  I  R  A  D  C  T  E  S  A  P  Y  A  V
5401 ATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTG
1821 *  N  T  A  Q  M  R  K  E  K  I  P  H  Q  A  L  F  R  F  L
5461 TGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTC
1841 A  H  *  L  A  A  L  G  R  S  A  A  A  S  G  I  S  S  L  K
5521 GCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAA
1861 G  G  N  T  V  I  H  R  I  R  G  *  R  R  K  E  H  V  S  K
5581 GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAA
1881 R  P  A  K  G  Q  E  P  *  K  G  R  V  A  G  V  F  P  *  A
5641 AGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT
1901 P  P  P  *  R  A  S  Q  K  S  T  L  K  S  E  V  A  K  P  D
5701 CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC
1921 R  T  I  K  I  P  G  V  S  P  W  K  L  P  R  A  L  S  C  S
5761 AGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCC
1941 D  P  A  A  Y  R  I  P  V  R  L  S  P  F  G  K  R  G  A  F
5821 GACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTC
1961 S  M  L  T  L  *  V  S  Q  F  G  V  G  R  S  L  Q  A  G  L
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5941 TGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGA
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6061 CAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
2041 H  *  K  D  S  I  W  Y  L  R  S  A  E  A  S  Y  L  R  K  K
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2081 Q  A  A  D  Y  A  Q  K  K  R  I  S  R  R  S  F  D  L  F  Y
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2221 S  C  N  F  I  R  L  H  P  V  Y  *  L  L  P  G  S  *  S  K
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FIGURE 26-5


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2561 A S * L K * R H F A R H G K I H N * E *
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2581 E S S D Q G Q E Q R N S * I P N R I S V
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His Asp Gly Gly Leu Ser Phe Gly Gly Ser Ser Gly Ile Trp Arg Pro
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Gly Ile Arg Pro Arg Gln Asp Met Trp Phe Trp Glu Thr Arg Thr Asn
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Pro His Arg Pro Gln Ser Arg Arg His Arg Ser Leu Asp Thr Arg Arg
420 425 430

Pro Arg Glu Gly Cys Arg Pro Arg Gly Trp Thr Ile Ser Arg Leu Thr
435 440 445

Arg Pro Leu Arg Thr Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu
450 455 460

Leu Ile Ser Ala Ser Val Ile Met Ser Arg Gly Ser Ile Val Met Thr
465 470 475 480

Gln Ser His Lys Phe Met Ser Thr Ser Val Gly Asp Ser Val Ser Ile
485 490 495

Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln
500 505 510

Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Ser Ala Ser Asp
515 520 525

Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
530 535 540

Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val
545 550 555 560

Tyr Tyr Cys His Gln His Tyr Ile Thr Pro Arg Thr Phe Gly Gly Gly
565 570 575

Thr Lys Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser
580 585 590

Glu Gly Lys Gly Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Met
595 600 605

Arg Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr
610 615 620

Phe Thr Arg Tyr Tyr Ile Gln Trp Gly Lys Asn Arg Pro Gly His Gly
625 630 635 640

Leu Glu Trp Ile Gly Glu Ile Leu Pro Gly Thr Leu Thr Asn Tyr Asn
645 650 655

Glu Lys Phe Lys Gly Lys Ala Ala Phe Thr Ala Asp Arg Ser Ser Asn
660 665 670

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
675 680 685

Tyr Tyr Cys Ala Arg Asp Gly Pro Trp Phe Ala Tyr Trp Gly Gln Gly
690 695 700

Thr Leu Val Thr Val Ser Ala Ala Asp Leu Ser Asn Ser Ile Met Tyr
705 710 715 720

Phe Ser His Phe Val Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Thr
725 730 735

Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro
740 745 750

Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val
755 760 765

His Thr Arg Gly Leu Asp Phe Ala Asp Pro Gln Val Gln Leu Gln Gln
770 775 780

Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys
785 790 795 800

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys
805 810 815

Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser
820 825 830

Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu
835 840 845

Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
850 855 860

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp
865 870 875 880

His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
885 890 895

Ser Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly Gln
900 905 910

Val Gln Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
915 920 925

Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
930 935 940

Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp
945 950 955 960

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg
965 970 975

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu
980 985 990

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro
995 1000 1005

Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg His His
1010 1015 1020

His His His His Thr Arg Gly Ser Ile Pro Pro Leu Ser Leu Pro
1025 1030 1035

Pro Pro Arg Tyr Trp Pro Lys Pro Leu Gly Ile Arg Pro Val Cys
1040 1045 1050

Val Cys Leu Tyr Val Ile Phe His His Ile Ala Val Phe Trp Gln
1055 1060 1065

Cys Glu Gly Pro Glu Thr Trp Pro Cys Leu Leu Asp Glu His Ser
1070 1075 1080

Gly Ser Phe Pro Ser Arg Gln Arg Asn Ala Arg Ser Val Glu Cys
1085 1090 1095

Arg Glu Gly Ser Ser Ser Ser Gly Ser Phe Leu Lys Thr Asn Asn
1100 1105 1110

Val Cys Ser Asp Pro Leu Gln Ala Ala Glu Pro Pro Thr Trp Arg
1115 1120 1125

Gln Val Pro Leu Arg Pro Lys Ala Thr Cys Ile Arg Tyr Thr Cys
1130 1135 1140

Lys Gly Gly Thr Thr Pro Val Pro Arg Cys Glu Leu Asp Ser Cys
1145 1150 1155

Gly Lys Ser Gln Met Ala Leu Leu Lys Arg Ile Gln Gln Gly Ala
1160 1165 1170

Glu Gly Cys Pro Glu Gly Thr Pro Leu Tyr Gly Ile Ser Gly Ala
1175 1180 1185

Ser Val His Met Leu Tyr Met Cys Leu Val Glu Val Lys Lys Arg
1190 1195 1200

Leu Gly Pro Pro Asn His Gly Asp Val Val Phe Leu Lys Thr Arg
1205 1210 1215

Tyr His Gly Asn Ser Arg Trp Ile Ala Arg Arg Phe Ser Gly Arg
1220 1225 1230

Leu Gly Gly Glu Ala Ile Arg Leu Leu Gly Thr Thr Asp Asn Arg
1235 1240 1245

Leu Leu Cys Arg Arg Val Pro Ala Val Ser Ala Gly Ala Pro Gly
1250 1255 1260

Ser Phe Cys Gln Asp Arg Pro Val Arg Cys Pro Glu Thr Ala Gly
1265 1270 1275

Arg Gly Ser Ala Ala Ile Val Ala Gly His Asp Gly Arg Ser Leu
1280 1285 1290

Arg Ser Cys Ala Arg Arg Cys His Ser Gly Lys Gly Leu Ala Ala
1295 1300 1305

Ile Gly Arg Ser Ala Gly Ala Gly Ser Pro Val Ile Ser Pro Cys
1310 1315 1320

Ser Cys Arg Glu Ser Ile His His Gly Cys Asn Ala Ala Ala Ala
1325 1330 1335

Tyr Ala Ser Gly Tyr Leu Pro Ile Arg Pro Pro Ser Glu Thr Ser
1340 1345 1350

His Arg Ala Ser Thr Tyr Ser Asp Gly Ser Arg Ser Cys Arg Ser

1355 1360 1365

Gly Ser Gly Arg Arg Ala Ser Gly Ala Arg Ala Ser Arg Thr Val
1370 1375 1380

Arg Gln Ala Gln Gly Ala His Ala Arg Arg Arg Gly Ser Arg Arg
1385 1390 1395

Asp Pro Trp Arg Cys Leu Leu Ala Glu Tyr His Gly Gly Lys Trp
1400 1405 1410

Pro Leu Phe Trp Ile His Arg Leu Trp Pro Ala Gly Cys Gly Gly
1415 1420 1425

Pro Leu Ser Gly His Ser Val Gly Tyr Pro Tyr Cys Arg Ala Trp
1430 1435 1440

Arg Arg Met Gly Pro Leu Pro Arg Ala Leu Arg Tyr Arg Arg Ser
1445 1450 1455

Arg Phe Ala Ala His Arg Leu Leu Ser Pro Ser Arg Val Leu Leu
1460 1465 1470

Ser Gly Thr Leu Gly Ile Arg Asn Lys Arg Phe Tyr Leu Val Ser
1475 1480 1485

Arg Lys Arg Gly Glu Lys Thr Pro Pro Val Gly Leu Ala Ser Leu
1490 1495 1500

Lys Arg His Phe Ala Arg His Gly Lys Ile His Asn Glu Arg Ser
1505 1510 1515

Ser Asp Gln Gly Gln Glu Gln Met Glu Gln Leu Asn Met Gly Gln
1520 1525 1530

Thr Gly Tyr Leu Trp Ala Val Pro Ala Pro Ala Gln Gly Gln Glu
1535 1540 1545

Gln Met Glu Gln Leu Asn Met Gly Gln Thr Gly Tyr Leu Trp Ala
1550 1555 1560

Val Pro Ala Pro Ala Gln Gly Gln Glu Gln Met Val Pro Arg Cys
1565 1570 1575

Gly Pro Ala Leu Ser Ser Phe Arg Thr Ile Arg Cys Phe Gln Gly
1580 1585 1590

Ala Pro Arg Thr Asn Asp Pro Val Pro Tyr Leu Asn Pro Ile Ser
1595 1600 1605

Ser Leu Leu Ala Ser Val Arg Ala Leu Leu Leu Pro Glu Leu Asn
1610 1615 1620

Lys Arg Ala His Asn Pro Ser Leu Gly Ala Pro Val Leu Arg Leu
1625 1630 1635

Thr Glu Ser Pro Gly Tyr Pro Cys Ile Gln Thr Leu Leu Gln Leu
1640 1645 1650

His Pro Thr Cys Gly Leu Ala Val Pro Trp Glu Gly Leu Leu Val
1655 1660 1665

Ile Asp Tyr Pro Ser Ala Gly Val Phe His Leu Gly Ala Arg Pro
1670 1675 1680

Gly Ser Gly Asp Pro Cys Pro Gly Thr Thr Asp Pro Pro Pro Gly
1685 1690 1695

Gly Lys Leu Ala Ala Ser Arg Val Ser Val Met Thr Val Lys Thr
1700 1705 1710

Ser Asp Thr Cys Ser Ser Arg Arg Arg Ser Gln Leu Val Cys Lys
1715 1720 1725

Arg Met Pro Gly Ala Asp Lys Pro Val Arg Ala Arg Gln Arg Val
1730 1735 1740

Leu Ala Gly Val Gly Ala Gln Pro Pro Ser His Val Ala Ile Ala
1745 1750 1755

Glu Cys Ile Leu Ala Leu Cys Gly Ile Arg Ala Asp Cys Thr Glu
1760 1765 1770

Ser Ala Pro Tyr Ala Val Asn Thr Ala Gln Met Arg Lys Glu Lys
1775 1780 1785

Ile Pro His Gln Ala Leu Phe Arg Phe Leu Ala His Leu Ala Ala
1790 1795 1800

Leu Gly Arg Ser Ala Ala Ala Ser Gly Ile Ser Ser Leu Lys Gly
1805 1810 1815

Gly Asn Thr Val Ile His Arg Ile Arg Gly Arg Arg Lys Glu His
1820 1825 1830

Val Ser Lys Arg Pro Ala Lys Gly Gln Glu Pro Lys Gly Arg Val
1835 1840 1845

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Ala Gly Val Phe Pro Ala Pro Pro Pro Arg Ala Ser Gln Lys Ser
    1850                1855                1860

Thr Leu Lys Ser Glu Val Ala Lys Pro Asp Arg Thr Ile Lys Ile
    1865                1870                1875

Pro Gly Val Ser Pro Trp Lys Leu Pro Arg Ala Leu Ser Cys Ser
    1880                1885                1890

Asp Pro Ala Ala Tyr Arg Ile Pro Val Arg Leu Ser Pro Phe Gly
    1895                1900                1905

Lys Arg Gly Ala Phe Ser Met Leu Thr Leu Val Ser Gln Phe Gly
    1910                1915                1920

Val Gly Arg Ser Leu Gln Ala Gly Leu Cys Ala Arg Thr Pro Arg
    1925                1930                1935

Ser Ala Arg Pro Leu Arg Leu Ile Arg Leu Ser Ser Val Gln Pro
    1940                1945                1950

Gly Lys Thr Arg Leu Ile Ala Thr Gly Ser Ser His Trp Gln Asp
    1955                1960                1965

Gln Ser Glu Val Cys Arg Arg Cys Tyr Arg Val Leu Glu Val Val
    1970                1975                1980

Ala Leu Arg Leu His Lys Asp Ser Ile Trp Tyr Leu Arg Ser Ala
    1985                1990                1995

Glu Ala Ser Tyr Leu Arg Lys Lys Ser Trp Leu Leu Ile Arg Gln
    2000                2005                2010

Thr Asn His Arg Trp Arg Trp Phe Phe Cys Leu Gln Ala Ala Asp
    2015                2020                2025

Tyr Ala Gln Lys Lys Arg Ile Ser Arg Arg Ser Phe Asp Leu Phe
    2030                2035                2040

Tyr Gly Val Arg Ser Val Glu Arg Lys Leu Thr Leu Arg Asp Phe
    2045                2050                2055

Gly His Glu Ile Ile Lys Lys Asp Leu His Leu Asp Pro Phe Lys
    2060                2065                2070

Leu Lys Met Lys Phe Ile Asn Leu Lys Tyr Ile Val Asn Leu Val
    2075                2080                2085
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Gln Leu Pro Met Leu Asn Gln Gly Thr Tyr Leu Ser Asp Leu Ser
2090 2095 2100

Ile Ser Phe Ile His Ser Cys Leu Thr Pro Arg Arg Val Asp Asn
2105 2110 2115

Tyr Asp Thr Gly Gly Leu Thr Ile Trp Pro Gln Cys Cys Asn Asp
2120 2125 2130

Thr Ala Arg Pro Thr Leu Thr Gly Ser Arg Phe Ile Ser Asn Lys
2135 2140 2145

Pro Ala Ser Arg Lys Gly Arg Ala Gln Lys Trp Ser Cys Asn Phe
2150 2155 2160

Ile Arg Leu His Pro Val Tyr Leu Leu Pro Gly Ser Ser Lys Phe
2165 2170 2175

Ala Ser Phe Ala Gln Arg Cys Cys His Cys Cys Arg His Arg Gly
2180 2185 2190

Val Thr Leu Val Val Trp Tyr Gly Phe Ile Gln Leu Arg Phe Pro
2195 2200 2205

Thr Ile Lys Ala Ser Tyr Met Ile Pro His Val Val Gln Lys Ser
2210 2215 2220

Gly Leu Leu Arg Ser Ser Asp Arg Cys Gln Lys Val Gly Arg Ser
2225 2230 2235

Val Ile Thr His Gly Tyr Gly Ser Thr Ala Phe Ser Tyr Cys His
2240 2245 2250

Ala Ile Arg Lys Met Leu Phe Cys Asp Trp Val Leu Asn Gln Val
2255 2260 2265

Ile Leu Arg Ile Val Tyr Ala Ala Thr Glu Leu Leu Leu Pro Gly
2270 2275 2280

Val Asn Thr Gly Tyr Arg Ala Thr Gln Asn Phe Lys Ser Ala His
2285 2290 2295

His Trp Lys Thr Phe Phe Gly Ala Lys Thr Leu Lys Asp Leu Thr
2300 2305 2310

Ala Val Glu Ile Gln Phe Asp Val Thr His Ser Cys Thr Gln Leu
2315 2320 2325

Ile Phe Ser Ile Phe Tyr Phe His Gln Arg Phe Trp Val Ser Lys

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    2330                2335                2340

Asn Arg  Lys Ala Lys Cys Arg  Lys Lys Gly Asn Lys  Gly Asp Thr
    2345                2350                2355

Glu Met  Leu Asn Thr His Thr  Leu Pro Phe Ser Ile  Leu Leu Lys
    2360                2365                2370

His Leu  Ser Gly Leu Leu Ser  His Glu Arg Ile His  Ile Met Tyr
    2375                2380                2385

Leu Glu  Lys Thr Asn Arg Gly  Ser Ala His Ile Ser  Pro Lys Ser
    2390                2395                2400

Ala Thr  Arg Leu Arg Asn His  Tyr Tyr His Asp Ile  Asn Leu Lys
    2405                2410                2415

Ala Tyr  His Glu Ala Leu Ser  Ser Ser Arg Ile His  Thr Arg Ser
    2420                2425                2430

Pro Lys  Thr Val Leu Gln Met  Cys Pro Pro His Thr  Pro Lys Phe
    2435                2440                2445

Ala Gly  Phe Cys Ser Thr Thr  Leu Pro Tyr Ser Pro  His Ser Pro
    2450                2455                2460

Glu Pro  Lys Pro Arg Pro Phe  Arg Phe Phe Ala Phe  Glu Arg Pro
    2465                2470                2475

His Pro  Val Ala Ser Leu Lys  Arg His Phe Ala Arg  His Gly Lys
    2480                2485                2490

Ile His  Asn Glu Glu Ser Ser  Asp Gln Gly Gln Glu  Gln Arg Asn
    2495                2500                2505

Ser Ile  Pro Asn Arg Ile Ser  Val Val Ser Gly Ser  Cys Pro Gly
    2510                2515                2520

Ser Gly  Pro Arg Thr Asp Glu  Thr Ala Glu Trp Ala  Lys Gln Asp
    2525                2530                2535

Ile Cys  Gly Lys Gln Phe Leu  Pro Arg Leu Gly Ala  Lys Asn Arg
    2540                2545                2550

Trp Ser  Pro Asp Ala Val Gln  Pro Ser Ala Val Ser  Ser Glu Ser
    2555                2560                2565

Ser Asp  Val Ser Arg Val Pro  Gln Gly Pro Glu Asn  Asp Pro Val
    2570                2575                2580
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Pro Tyr Leu Asn Pro Ile Ser Ser Leu Leu Ala Ser Val Arg Ala
2585 2590 2595

Leu Pro Leu Ser Glu Leu Asn Lys
2600 2605

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<212> DNA
<213> Artificial Sequence

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<223> E3Bi cDNA Sequence

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agagggagca ttgtaatgac ccaatctcac aaattcatgt ccacatcagt aggagacagt 120

gtcagcatca cctgcaaggc cagtcaggat gtgagtactg ctgtagcctg gtatcaacag 180

aaaccaggac aatctcctaa actactgatt tactcggcat ccgaccggta cactggagtc 240

cctgatcgct tcactggcag tggatctggg acggatttca ctttcaccat cagcagtgtg 300

caggctgaag acctggcagt ttattactgt caccaacatt atattactcc tcggacgttc 360

ggtggaggca caaagctgga aataaaaggg tcgacttccg gtagcggcaa atcctctgaa 420

ggcaaaggtc aggtccagct gcagcagtct ggagctgagg tgatgaggcc tggggcctca 480

gtgaagatat cctgcaaggc tactggctac acattcacta ggtactacat acaatggggt 540

aaaaacaggc ctggacatgg ccttgagtgg attggagaga ttttacctgg aactcttact 600

aattacaatg agaaattcaa gggcaaggcc gcattcactg cagatagatc ctccaacaca 660

gcctacatgc aactcagcag ccttacatct gaggactctg ccgtctatta ctgtgcaaga 720

gatggtccct ggtttgctta ctggggccaa ggaaccctgg tcaccgtctc tgcagcggat 780

ctgagcaact ccatcatgta cttcagccac ttcgtgccgg tcttcctgcc agcgaagccc 840

accacgacgc cagcgccgcg accaccaaca ccggcgccca ccatcgcgtc gcagcccctg 900

tccctgcgcc cagaggcgtg ccggccagcg gcggggggcg cagtccacac gagggggctg 960

gacttcgcgg atccacaggt ccagctacag cagtctgggg ctgaactggc aagacctggg 1020

gcctcagtga agatgtcctg caaggcttct ggctacacct ttactaggta cacgatgcac 1080

tgggtaaaac agaggcctgg acagggtctg gaatggattg gatacattaa tcctagccgt 1140

ggttatacta attacaatca gaagttcaag gacaaggcca cattgactac agacaaatcc 1200

tccagcacag cctacatgca actgagcagc ctgacatctg aggactctgc agtctattac 1260

tgtgcaagat attatgatga tcattactgc cttgactact ggggccaagg caccactctc 1320

acagtctcct caggatctac ttcaggtagc ggtaaatcat ctgaaggtaa aggtcaggtc 1380

ctccaaattg ttctcaccca gtctccagca atcatgtctg catctccagg ggagaaggtc 1440

accatgacct gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca 1500

ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg agtccctgct 1560

cacttcaggg gcagtgggtc tgggacctct tactctctca caatcagcgg catggaggct 1620

gaagatgctg ccacttatta ctgccagcag tggagtagta acccattcac gttcggctcg 1680

gggacaaagt tggaaataaa ccggcaccat caccatcacc attagactcg a 1731

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<211> 574
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein Sequence of E3Bi

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Val Ile Met Ser Arg Gly Ser Ile Val Met Thr Gln Ser His Lys Phe
            20   25   30

Met Ser Thr Ser Val Gly Asp Ser Val Ser Ile Thr Cys Lys Ala Ser
        35   40   45

Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln
    50   55   60

Ser Pro Lys Leu Leu Ile Tyr Ser Ala Ser Asp Arg Tyr Thr Gly Val
65   70   75   80

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
                85   90   95

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
            100   105   110

His Tyr Ile Thr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        115   120   125

Lys Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly Gln
    130   135   140

Val Gln Leu Gln Gln Ser Gly Ala Glu Val Met Arg Pro Gly Ala Ser
145   150   155   160

Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Thr Arg Tyr Tyr
                165   170   175

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Ile Gln Trp Gly Lys Asn Arg Pro Gly His Gly Leu Glu Trp Ile Gly
            180                 185                 190

Glu Ile Leu Pro Gly Thr Leu Thr Asn Tyr Asn Glu Lys Phe Lys Gly
        195                 200                 205

Lys Ala Ala Phe Thr Ala Asp Arg Ser Ser Asn Thr Ala Tyr Met Gln
    210                 215                 220

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
225                 230                 235                 240

Asp Gly Pro Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
                245                 250                 255

Ser Ala Ala Asp Leu Ser Asn Ser Ile Met Tyr Phe Ser His Phe Val
            260                 265                 270

Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Thr Pro Ala Pro Arg Pro
        275                 280                 285

Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro
    290                 295                 300

Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu
305                 310                 315                 320

Asp Phe Ala Asp Pro Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
                325                 330                 335

Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
            340                 345                 350

Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln
        355                 360                 365

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn
    370                 375                 380

Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser
385                 390                 395                 400

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
                405                 410                 415

Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp
            420                 425                 430
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Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Ser Thr Ser
435 440 445

Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly Gln Val Leu Gln Ile Val
450 455 460

Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val
465 470 475 480

Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
485 490 495

Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser
500 505 510

Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly
515 520 525

Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala
530 535 540

Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser
545 550 555 560

Gly Thr Lys Leu Glu Ile Asn Arg His His His His His His
565 570

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<211> 2606
<212> PRT
<213> Artificial Sequence

<220>
<223> Alternative Protein Sequence of pG1EN-EH3.His (E3-Bi and Vector)

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Ala Arg Val Pro Val Phe Pro Ile Lys Pro Leu Ala Val Cys Ile Arg
20 25 30

Ile Val Val Ser Leu Phe Leu Gly Arg Val Ser Ser Glu Leu Thr Thr
35 40 45

His Asp Gly Gly Leu Ser Phe Gly Gly Ser Ser Gly Ile Trp Arg Pro
50 55 60

Leu Pro Arg Asp His Arg Pro Thr Thr Gly Arg Ala Gly Gln Gln Pro
65 70 75 80

Ile Cys Val Cys Pro Ile Val Cys Leu Cys Leu Met Leu Cys Ala Cys
85 90 95

Val Cys Thr Ser Leu Thr Ser Ser Val Ser Gly Gly Pro Val Val Glu
100 105 110

Leu Thr Ser Ser Glu His Pro Ala Ala Thr Gln Gly Asp Val Pro Gly
115 120 125

Thr Leu Gly Ala Val Phe Val Ala Arg Pro Glu Glu Gly Ser Arg Cys
130 135 140

Gly Ile Arg Pro Arg Gln Asp Met Trp Phe Trp Glu Thr Arg Thr Asn
145 150 155 160

Ser Ser Arg Leu Arg Leu Asn Phe Cys Phe Arg Phe Gly Thr Glu Ala
165 170 175

Ala Arg Leu Val Cys Cys Ser Ile Val Leu Cys Cys Leu Cys Leu Thr
180 185 190

Val Phe Leu Tyr Leu Ser Glu Asn Gly Gln Thr Val Thr Thr Pro Leu
195 200 205

Ser Leu Thr Leu Gly His Trp Lys Asp Val Glu Arg Ile Ala His Asn
210 215 220

Gln Ser Val Asp Val Lys Lys Arg Arg Trp Val Thr Phe Cys Ser Ala
225 230 235 240

Glu Trp Pro Thr Phe Asn Val Gly Trp Pro Arg Asp Gly Thr Phe Asn
245 250 255

Arg Asp Leu Ile Thr Gln Val Lys Ile Lys Val Phe Ser Pro Gly Pro
260 265 270

His Gly His Pro Asp Gln Val Pro Tyr Ile Val Thr Trp Glu Ala Leu
275 280 285

Ala Phe Asp Pro Pro Pro Trp Val Lys Pro Phe Val His Pro Lys Pro
290 295 300

Pro Pro Pro Leu Pro Pro Ser Ala Pro Ser Leu Pro Leu Glu Pro Pro
305 310 315 320

Arg Ser Thr Pro Pro Arg Ser Ser Leu Tyr Pro Ala Leu Thr Pro Ser
325 330 335

Leu Gly Ala Gly Ile Arg Gly Arg Asp Lys Ser Tyr Gln Pro Leu Ser
340 345 350

Pro Ser Ser Leu Thr Gly Ser Leu Leu Ser Pro Ala Arg Ser Leu Glu
355 360 365

Thr Ser Gly Gly Ser Leu Pro Arg Thr Thr Gly Pro Thr Gly Gly Thr
370 375 380

Ser Pro Leu Pro Ser Arg Arg His Ser Val Gly Pro Pro Thr Pro Asp
385 390 395 400

Glu Pro Arg Thr Ser Leu Glu Arg Thr Leu His Ser Pro Ala Asp His
405 410 415

Pro His Arg Pro Gln Ser Arg Arg His Arg Ser Leu Asp Thr Arg Arg
420 425 430

Pro Arg Glu Gly Cys Arg Pro Arg Gly Trp Thr Ile Ser Arg Leu Thr
435 440 445

Arg Pro Leu Arg Thr Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu
450 455 460

Leu Ile Ser Ala Ser Val Ile Met Ser Arg Gly Ser Ile Val Met Thr
465 470 475 480

Gln Ser His Lys Phe Met Ser Thr Ser Val Gly Asp Ser Val Ser Ile
485 490 495

Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln
500 505 510

Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Ser Ala Ser Asp
515 520 525

Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
530 535 540

Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val
545 550 555 560

Tyr Tyr Cys His Gln His Tyr Ile Thr Pro Arg Thr Phe Gly Gly Gly
565 570 575

Thr Lys Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser
580 585 590

Glu Gly Lys Gly Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Met
595 600 605

Arg Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr
610 615 620

Phe Thr Arg Tyr Tyr Ile Gln Trp Gly Lys Asn Arg Pro Gly His Gly
625 630 635 640

Leu Glu Trp Ile Gly Glu Ile Leu Pro Gly Thr Leu Thr Asn Tyr Asn
645 650 655

Glu Lys Phe Lys Gly Lys Ala Ala Phe Thr Ala Asp Arg Ser Ser Asn
660 665 670

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
675 680 685

Tyr Tyr Cys Ala Arg Asp Gly Pro Trp Phe Ala Tyr Trp Gly Gln Gly
690 695 700

Thr Leu Val Thr Val Ser Ala Ala Asp Leu Ser Asn Ser Ile Met Tyr
705 710 715 720

Phe Ser His Phe Val Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Thr
725 730 735

Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro
740 745 750

Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val
755 760 765

His Thr Arg Gly Leu Asp Phe Ala Asp Pro Gln Val Gln Leu Gln Gln
770 775 780

Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys
785 790 795 800

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys
805 810 815

Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser
820 825 830

Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu
835 840 845

Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu

850 855 860

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp
865 870 875 880

His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
885 890 895

Ser Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly Gln
900 905 910

Val Gln Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
915 920 925

Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
930 935 940

Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp
945 950 955 960

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg
965 970 975

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu
980 985 990

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro
995 1000 1005

Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg His His
1010 1015 1020

His His His His Thr Arg Gly Ser Ile Pro Pro Leu Ser Leu Pro
1025 1030 1035

Pro Pro Arg Tyr Trp Pro Lys Pro Leu Gly Ile Arg Pro Val Cys
1040 1045 1050

Val Cys Leu Tyr Val Ile Phe His His Ile Ala Val Phe Trp Gln
1055 1060 1065

Cys Glu Gly Pro Glu Thr Trp Pro Cys Leu Leu Asp Glu His Ser
1070 1075 1080

Gly Ser Phe Pro Ser Arg Gln Arg Asn Ala Arg Ser Val Glu Cys
1085 1090 1095

Arg Glu Gly Ser Ser Ser Ser Gly Ser Phe Leu Lys Thr Asn Asn
1100 1105 1110

Val Cys Ser Asp Pro Leu Gln Ala Ala Glu Pro Pro Thr Trp Arg
1115 1120 1125

Gln Val Pro Leu Arg Pro Lys Ala Thr Cys Ile Arg Tyr Thr Cys
1130 1135 1140

Lys Gly Gly Thr Thr Pro Val Pro Arg Cys Glu Leu Asp Ser Cys
1145 1150 1155

Gly Lys Ser Gln Met Ala Leu Leu Lys Arg Ile Gln Gln Gly Ala
1160 1165 1170

Glu Gly Cys Pro Glu Gly Thr Pro Leu Tyr Gly Ile Ser Gly Ala
1175 1180 1185

Ser Val His Met Leu Tyr Met Cys Leu Val Glu Val Lys Lys Arg
1190 1195 1200

Leu Gly Pro Pro Asn His Gly Asp Val Val Phe Leu Lys Thr Arg
1205 1210 1215

Tyr His Gly Asn Ser Arg Trp Ile Ala Arg Arg Phe Ser Gly Arg
1220 1225 1230

Leu Gly Gly Glu Ala Ile Arg Leu Leu Gly Thr Thr Asp Asn Arg
1235 1240 1245

Leu Leu Cys Arg Arg Val Pro Ala Val Ser Ala Gly Ala Pro Gly
1250 1255 1260

Ser Phe Cys Gln Asp Arg Pro Val Arg Cys Pro Glu Thr Ala Gly
1265 1270 1275

Arg Gly Ser Ala Ala Ile Val Ala Gly His Asp Gly Arg Ser Leu
1280 1285 1290

Arg Ser Cys Ala Arg Arg Cys His Ser Gly Lys Gly Leu Ala Ala
1295 1300 1305

Ile Gly Arg Ser Ala Gly Ala Gly Ser Pro Val Ile Ser Pro Cys
1310 1315 1320

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1325 1330 1335

Tyr Ala Ser Gly Tyr Leu Pro Ile Arg Pro Pro Ser Glu Thr Ser
1340 1345 1350

His Arg Ala Ser Thr Tyr Ser Asp Gly Ser Arg Ser Cys Arg Ser
1355 1360 1365

Gly Ser Gly Arg Arg Ala Ser Gly Ala Arg Ala Ser Arg Thr Val
1370 1375 1380

Arg Gln Ala Gln Gly Ala His Ala Arg Arg Arg Gly Ser Arg Arg
1385 1390 1395

Asp Pro Trp Arg Cys Leu Leu Ala Glu Tyr His Gly Gly Lys Trp
1400 1405 1410

Pro Leu Phe Trp Ile His Arg Leu Trp Pro Ala Gly Cys Gly Gly
1415 1420 1425

Pro Leu Ser Gly His Ser Val Gly Tyr Pro Tyr Cys Arg Ala Trp
1430 1435 1440

Arg Arg Met Gly Pro Leu Pro Arg Ala Leu Arg Tyr Arg Arg Ser
1445 1450 1455

Arg Phe Ala Ala His Arg Leu Leu Ser Pro Ser Arg Val Leu Leu
1460 1465 1470

Ser Gly Thr Leu Gly Ile Arg Asn Lys Arg Phe Tyr Leu Val Ser
1475 1480 1485

Arg Lys Arg Gly Glu Lys Thr Pro Pro Val Gly Leu Ala Ser Leu
1490 1495 1500

Lys Arg His Phe Ala Arg His Gly Lys Ile His Asn Glu Arg Ser
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Ser Asp Gln Gly Gln Glu Gln Met Glu Gln Leu Asn Met Gly Gln
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Thr Gly Tyr Leu Trp Ala Val Pro Ala Pro Ala Gln Gly Gln Glu
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Gln Met Glu Gln Leu Asn Met Gly Gln Thr Gly Tyr Leu Trp Ala
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Val Pro Ala Pro Ala Gln Gly Gln Glu Gln Met Val Pro Arg Cys
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Ala Pro Arg Thr Asn Asp Pro Val Pro Tyr Leu Asn Pro Ile Ser
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Lys Arg Ala His Asn Pro Ser Leu Gly Ala Pro Val Leu Arg Leu
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Thr Glu Ser Pro Gly Tyr Pro Cys Ile Gln Thr Leu Leu Gln Leu
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His Pro Thr Cys Gly Leu Ala Val Pro Trp Glu Gly Leu Leu Val
1655 1660 1665

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1775 1780 1785

Ile Pro His Gln Ala Leu Phe Arg Phe Leu Ala His Leu Ala Ala
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1805 1810 1815

Gly Asn Thr Val Ile His Arg Ile Arg Gly Arg Arg Lys Glu His
1820 1825 1830

Val Ser Lys Arg Pro Ala Lys Gly Gln Glu Pro Lys Gly Arg Val

1835 1840 1845

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1850 1855 1860

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1865 1870 1875

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1880 1885 1890

Asp Pro Ala Ala Tyr Arg Ile Pro Val Arg Leu Ser Pro Phe Gly
1895 1900 1905

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1910 1915 1920

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Ser Ala Arg Pro Leu Arg Leu Ile Arg Leu Ser Ser Val Gln Pro
1940 1945 1950

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1955 1960 1965

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1970 1975 1980

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1985 1990 1995

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2000 2005 2010

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2015 2020 2025

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2045 2050 2055

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Leu Lys Met Lys Phe Ile Asn Leu Lys Tyr Ile Val Asn Leu Val
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Pro Ala Ser Arg Lys Gly Arg Ala Gln Lys Trp Ser Cys Asn Phe
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2210 2215 2220

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Val Asn Thr Gly Tyr Arg Ala Thr Gln Asn Phe Lys Ser Ala His
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2525 2530 2535

Ile Cys Gly Lys Gln Phe Leu Pro Arg Leu Gly Ala Lys Asn Arg
2540 2545 2550

Trp Ser Pro Asp Ala Val Gln Pro Ser Ala Val Ser Ser Glu Ser
2555 2560 2565

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Ser Asp  Val Ser Arg Val Pro  Gln Gly Pro Glu Asn  Asp Pro Val
    2570                 2575                 2580

Pro Tyr  Leu Asn Pro Ile Ser  Ser Leu Leu Ala Ser  Val Arg Ala
    2585                 2590                 2595

Leu Pro  Leu Ser Glu Leu Asn  Lys
    2600                 2605
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(71) Applicant (*for all designated States except US*): ROGER WILLIAMS HOSPITAL [US/US]; 825 Chalkstone Avenue, Providence, RI 02908 (US).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): REN-HEIDENREICH, Lifen [US/US]; 62 Tiffany Road, Coventry, RI 02816 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/090513 A3

(54) Title: BI-SPECIFIC ANTIGEN-BINDING COMPOSITIONS AND RELATED METHODS

(57) Abstract: This invention provides a composition of matter comprising a first antigen-binding moiety and a second antigen-binding moiety operably affixed to one another via a flexible linker moiety. This invention also provides related nucleic acids, host-vector systems, compositions and methods of polypeptide production. This invention further provides related methods of treating a subject afflicted with a disorder mediated by the presence of an abnormal cell, and kits for practicing same.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/12772

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07K 16/46; C12N 15/62
US CL : 424/136.1, 69.7; 435/328, 7.1; 530/387.3, 412, 413; 536/23.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/136.1, 69.7; 435/328, 7.1; 530/387.3, 412, 413; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,837, 242 A (HOLLINGER et al) 17 November. 1998 (17.11.1998), see columns 1-8, 13-16, 20-28 and example 18.	1-3, 9-13, 19, 20-23, 38 ----------- 4-7, 24, 27-35, 51, 52.
X --- Y	MATTHIAS M. et al. A small bispecific antibody construct expressed as a functional single-chain molecule with high tumor cell cytotoxicity. Proc. Natl. Acad. Sci. USA. July 1995, Vol. 92, pages 7021-7025. see entire document.	1-3, 10-14, 19, 22, 34, 35, 38. ----------- 39-43.
X	MERTENS N. et al. New recombinant bi- and trispecific antibody derivatives. Novel Frontiers in the Production of Compounds for Biomedical Use. 2001, Kluwer Academic Publishers, pages 195-208. see entire documemt.	1-5, 9, 10-13, 19-20, 22-24, 27-30, 32-35, 38.
X --- Y	FLEIGER D. et al. A bispecific single-chain anitbody directed against EpCAM/CD3 in combination with the cytokines interferon alpha and interleukin-2 efficiently retargets T and CD3+CD56+ natural-killer-like T lymphocytes to EpCAM-expressing tumor cells. Cancer Immunol. Immunother. 2000, Vol. 49 pages 441-448. See entire document.	1-3, 10-15, 19-20, 22, 34, 31-3, 10-15, 19-20, 22, 34-35, 38. ----------- 39-43, 46-51, 55-57.

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
03 October 2003 (03.10.2003)

Date of mailing of the international search report
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Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Valerie Bell-Harris for
David J. Blanchard
Telephone No. (703) 308-0196

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PCT/US03/12772

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOLHUIS R. L. H. et al. Adoptive immuntherapy of ovarian carcinoma with BS-MAb-targeted lymphocytes: A multicellular study. 1992, Vol. 7, pages 78-81. See pages 79-80.	39-43, 46-51, 55-57.

Continuation of B. FIELDS SEARCHED Item 3:

Sequence search databases: SEQ ID Nos. 1-4, Geneseq, Swissprot, Sptrembl, patents, EST, GenEmbl, Medline, WEST, Biosis.
Search terms: diabody, CD3, EpCAM, bi-specific antibody, single-chain antibody, tumor antigen, TAA, multivalent/multispecific binding proteins, 17-1A, OkT3 antibody, GA733.2 antibody, E3bi antibody.